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Studies on the excretion of porphobilinogen in patients with so-called acute porphyria.

By

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It is indeed an interesting fact that heredity seems to play a dominant part in the development of most of the less common disturbances of metabolism. Such instances as the two »forms» of diabetes insipidus, (Veil simple, dominant; Forssman recessive, sex linked), pentosuria (recessive), alcaptonuria (recessive), cystinuria (dominant or recessive), familial idiopathic methemoglobinemia, Bensley, Rhea and Mills, and different forms of lipoidosis may be quoted (»inborn errors of metabolism», Garrod). It seems pretty certain, that several of the hereditary diseases of the nervous system are also associated with, possibly caused by, metabolic disturbances (cf. juvenile amaurotic idiocy and the imbecillitas phenylpyrrouvica described by Fölling).

So-called acute porphyria with its manifold symptoms from the central and peripheral nervous system may well be regarded as belonging to this group as will be shown later in this paper. As a matter of fact the name acute porphyria is not very appropriate, since it has been shown that the malady is a lifelong chronic derangement of pyrrole, but not necessarily of porphyrin metabolism, with acute episodes (Waldenström).

It has long been known that another metabolic disorder, the congenital porphyrinuria with light sensitivity, might occur among several members of the same family. Arzt and Hausmann saw two

brothers affected with the malady, whose parents were first cousins (see also Mc Call Anderson, Ehrmann, Hans Fischer etc.). This character is almost certainly recessive.

The work of Waldenström (1937) has made it clear that so-called acute porphyria occurs as a dominant character and cases with light sensitivity, which is the most important symptom in congenital porphyria, were never found in the large number of families with acute porphyria examined by him. There is thus no genetical connection between the two so-called »forms» of porphyria. There are at present known about 150 cases of acute porphyria from Sweden but not a single case of congenital porphyria. They are two distinct and separate maladies.

Acute porphyria is no very rare malady in Sweden. In 1937 Waldenström collected 103 instances from Sweden. From this time the number of cases has increased considerably and at present the number of known cases in Sweden is about 150. This increase is chiefly due to the investigations of brothers and sisters and other nearer relatives of porphyrics. The actual number of new cases with severe symptoms is also rather high and it is evident, that the malady may be found much more often than was previously realized, if it is correctly diagnosed.

The question of heredity was investigated by Waldenström in 1937 and it was shown that in the large majority of patients with acute porphyria the disease is familial. Later investigations have shown, that among the 103 instances of porphyria only 12 are as yet to be regarded as isolated and among these it has only been possible to examine samples of urine from a number of brothers and sisters in 4. In the other 8 closer investigations were impossible from external reasons (an only child, parents dead or unknown etc.). It is thus obvious, that heredity plays a dominant part for the development of acute porphyria.

The unpublished results of Engel and Waldenström show that a very large majority of the Swedish cases belong to the same family with no less than 100 members suffering from porphyria. In nearly every case from the North of Sweden has it been possible to trace a connection with this large family of porphyrics or with two other families of lap origin (21 porphyric members).

The present paper deals with problems regarding the metabolism of some pyrrole derivatives in acute porphyria. The work of

Waldenström and Vahlquist (1939) has proved that in this condition porphyrin as such is probably never excreted primarily through the kidney. It is formed in the urine through the condensation of two molecules of porphobilinogen, as we have called the colourless substance (the chromogen of Waldenström) always occurring in the urine in acute porphyria. This condensation largely depends upon external factors such as temperature and reaction of the urine. Two molecules of porphobilinogen, each containing two pyrrole nuclei may either form a ring or a chain. In the first case a porphyrin is formed, in the latter a urobilinoid pigment, which we have called porphobilin. The fact that porphyrin has never been demonstrated in the blood of acute porphyrics (but several times in congenital porphyria) is thus easily explained. In this malady only porphobilinogen in large amounts is formed inside the organism. Many authors have in vain tried to obtain any signs of light sensitivity in their patients with acute porphyria and this question has been a matter of considerable discussion. It is quite evident that the non-fluorescent porphobilinogen cannot act as a sensitizer against the light rays.

Under these circumstances the quantitative determination of the porphobilinogen excreted in the urine may give an opportunity to follow the change in the metabolic disorder, that has been called acute porphyria. Several observers (among others Vannotti) have tried to make estimations of the amount of uroporphyrin in the urine. We have been able to show that the amount of porphyrin formed from the porphobilinogen is largely dependant on accidental factors. Even though there now exists a reliable method for the isolation of uroporphyrin III from the urine (extraction with ethyl acetate at P_H 3.2 according to Waldenström) the method is mainly important for the preparation of this porphyrin. For the investigation of the functional pathology of acute porphyria only the determination of porphobilinogen in the urine is of any real value. There may however be found a very slight amount of porphyrin already in the freshly voided, acid urine. It is probably formed in the bladder and its amount is quite insignificant as compared with the porphobilinogen. One of us (Vahlquist 1939) has worked out a method for the quantitative estimation of porphobilinogen in solutions. It has been used in the present investigation.

Exogenous factors influencing the excretion of porphobilinogen.

The principal question to be answered as regards the functional pathology in this type of disease is the following. Is the formation or excretion of the pathological metabolite influenced by exogenous factors such as the administration of certain substances that may be supposed to be related chemically to this metabolite. We know for example that tyrosin given to an alcaptonuric greatly increases the output of homogentisic acid. The same holds true for the excretion of phenylpyruvic acid after administration of phenylalanin in Fölling's disease (Jervis). It must therefore be assumed, that the ingested substances are closely related to those excreted and that the latter are possibly formed by a change in the composition of the former.

The same problem ought to be of great importance for the comprehension of the mechanism at work in acute porphyria. Many facts seem to favour the assumption, that we have to deal with a disturbed synthesis of porphyrins. It seems a priori possible, that the less complex pyrrole derivatives related to pyrrole such as proline and oxyproline may be used by the organism for the synthesis of porphobilinogen and also for the formation of the prosthetic group in hemoglobin.

We have had the opportunity to investigate some of these problems in two cases of acute porphyria. In nine others were we able to make quantitative determinations of porphobilinogen on freshly voided specimens of urine.

The first case is a man, born in 1892. He belongs to family XII in Waldenström's monograph on acute porphyria. His urine had not previously been examined. He had been suffering from a duodenal ulcer for many years and had been operated upon for that malady. He now showed definite clinical and radiological signs of a peptic ulcer and it was regarded advisable to give him a severe régime for ulcer. During the first two days of his stay in the hospital he got the usual mixed diet for a patient with gastric ulcer. For one day he was then fasting and on the following four days he was only taking increasing quantities of a mixture containing cream and water on parts. During this time his food intake may consequently be regarded as very low in protein. The patient now began to get symptoms possibly indicating an imminent attack of acute porphyria. His fluid intake was therefore considerably increased and his diet was less and less restricted. At the end of his hospital time (3 weeks) his previous dyspeptic troubles had disappeared and he was dismissed very

much improved. As a matter of fact he was not ordered barbiturates of any kind during his stay in the hospital. (cf. Waldenström, 1939).

From Fig. 1 it is clearly seen that the *absolute amount* of excreted porphobilinogen is not at all decreased during the low protein diet. The concentration of porphobilinogen in the urine is unusually high during the same time, a fact that is probably explained by the limitation of fluid intake. The concentration reached 20–30 P. U. (P. U. = porphobilinogen-unit)/100 cm³ (see Vahlquist). Later the concentration remains fairly constant about 7–10 P. U./100 cm³. During the period of fasting the patient developed marked acidosis. The colour of the urine was reddish during this time and darkened very quickly after micturition. Treatment

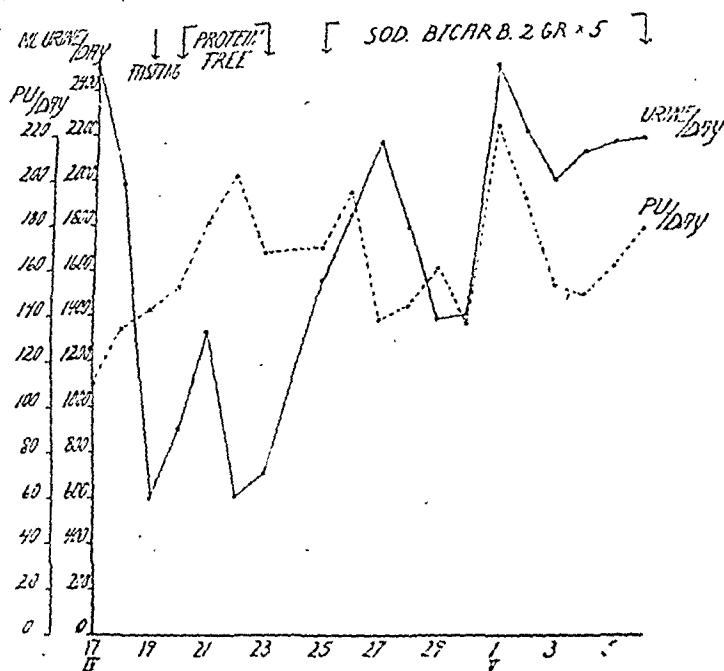


Fig. 1.

with 5 gms sodium bicarbonate thrice daily had an immediate effect. The acidity decreased and the colour of the urine became absolutely normal. There was no darkening on standing. It is seen from the fig. that the amount of excreted porphobilinogen is very much the same during both periods. When the light yellow urine was acidified and boiled it assumed a dark red colour and the bands of uroporphyrin and porphobilin were clearly to be seen.

Our next patient was case 64 (Waldenström, 1937). She had been suffering from colics and very severe constipation the last weeks before admittance. Also her psychic balance was considerably disturbed as her muscular power had decreased very rapidly during the last week and she was fearing a relapse of her paralysis. The first days we treated her with prostigmin injections and also with repeated duodenal tubage. Our thought was that toxic substances might possibly be excreted through the bile,

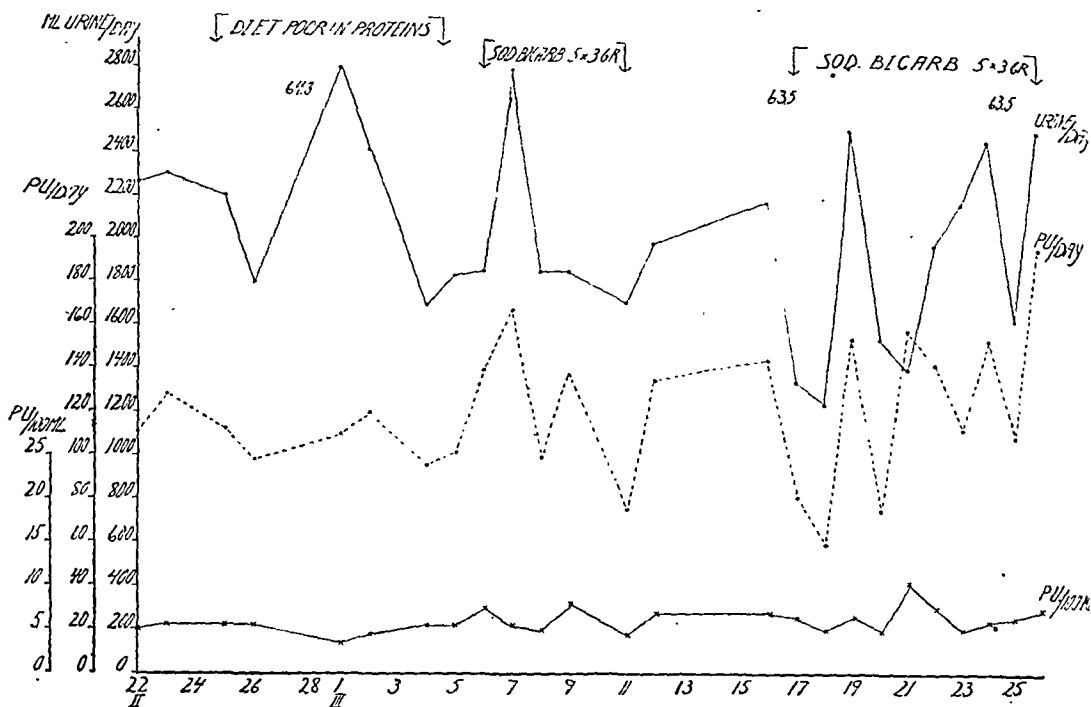


Fig. 2.

and their reabsorption from the bowel favoured by the longstanding constipation. It is not easy to say if this assumption is correct but the patient's general status improved considerably with this treatment and the signs of «intoxication» soon disappeared. She was not allowed any barbiturics during this time (Waldenström 1939).

The first week she was put on a mixed diet. It is seen that the daily excretion of porphobilinogen varied but little during this time kept about 100—120 P. U./24 hrs. or 5—7 P. U./100 cm³. The reaction of the urine during this time was rather acid with a P_H of about 5.5. The urine darkened quickly after voiding. — The administration of sodium bicarbonate 5 gms thrice daily did not appreciably alter the output of porphobilinogen. In spite of this the urine kept its normal yellow appearance indefinitely on standing. Boiling the acidified urine however at once transformed the porphobilinogen into porphyrin and porphobilin. (See colour photograph at the end of this paper). The administration of bicarbonate was then stopped, the urine once again became acid and darkened on standing. A new period of alkali therapy had the same influence as the first one. (Fig. 2)

The condition of the patient did not make it justified to put her on a diet that might provoke an acidosis and possibly also induce a porphyric attack. For somewhat more than a week however she was put on a low protein diet. It chiefly consisted of carbohydrates in the form of sugar,

strongly sweetened fruit juices, jams and so on. She got practically no bread but some vegetables cooked with much butter. On this diet which was very low in protein content without causing an acidosis there was no apparent change in porphobilinogen output. (Fig. 3)

It is pretty clear from the figures reproduced here that the excretion of porphobilinogen is not appreciably influenced by changes in the protein i. e. also the prolin and oxyprolin intake. It seems probable that it is an endogenous product of metabolism. It ought to be especially emphasized that not even a period of fasting followed by a practically pure intake of fat and <4 for 5 days brought about any decrease in porphobilinogen excretion. In this respect acute porphyria seems to be fundamentally different

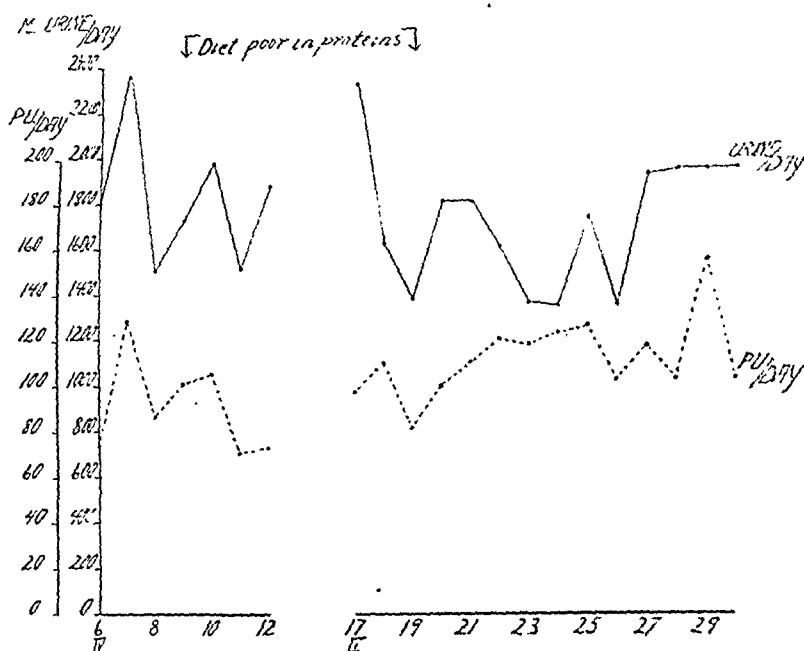


Fig. 3.

from such inborn errors of metabolism as alcaptonuria and Fölling's disease. Another important difference is purely quantitative. Whereas homogentisic and phenylpyruvic acid are excreted in grammes daily the excretion of porphobilinogen may probably be measured in milligrammes.

It is to be regretted, that our knowledge of the connection between porphobilinogen and other substances of the animal organism has not been furthered through these experiments. The negative conclusion however that such pyrrole-containing substances in the food as hemo- and myoglobin, chlorophyll and proline can hardly be regarded as sources for the formation of porphobilinogen seems justifiable.

Regarding the histories of the cases a few words ought to be said. Case III belongs to family XVI, and she was in 1937 regarded as possibly porphyric although her urine had not been examined. She was sent down to the Medical Clinic in Upsala with the diagnosis acute porphyria. Her

Table 1.

Case	Vol. of urine ml/24 hrs.	Spec. grav.	P. U./100 ml.		P. U./24 hrs.		Stage of disease
			directly	reduced fosp. gr. 1.015	total	pro 10 kg of body weight	
I	1380		11.0		150	$150/7.7 = 19.5$	Recent porphyric attack.
II	2400	1,012	4.9	5.2	117	$117/6.5 = 18.0$	Recent porphyric attack.
III	800	1,021	10.2	7.3	81	$81/4.5 = 18.0$	Probably no porphyric attack. Fever caused by urinary infection.
IV	625	1,018	10.8	9.0	68		Recent porphyric attack.
V	750	1,019	7.6	6.0	57		Recent porphyric attack.
VI	—	1,025	10.4	6.2	—		Recent porphyric attack.
VII	—	1,036	8.6	3.6	—		Latent porphyria.
VIII	—	1,030	1.4	0.7	—		" "
IX	—	1,022	4.4	3.0	—		" "
X	—	1,032	6.2	2.9	—		" "
XI	—	1,015	2.0	2.0	—		" "

clinical history however was not in any way typical of acute porphyria, and it was found that she had a severe urinary infection. Sometimes fecal matters seemed to be passed with the urine. The tentative diagnosis of *fistula recto-vesicalis* was made. X-ray pictures failed to demonstrate any connection when the bladder was filled but from an opaque enema there could be seen a fine fistula opening into the bladder. At the operation she was found to have bilateral pyosalpinx and there was in reality a *fistula recto-salpingo-vesicalis*. The patient died a few days after the operation. We do not think, that she ever had any signs of a porphyric attack but it seems probable, that her metabolic disorder was aggravated by her chronic infection with fever. The case illustrates very well the obvious but practically important fact that a latent porphyric may suffer from another acute abdominal malady than a porphyric colic.

Case IV had a typical acute severe porphyria with extensive pareses. Case V and VI were only suffering from abdominal colics and a rather severe intoxication but without any involvement of the nervous system. Cases VII—XI were typical latent porphyrics without subjective symptoms. The table seems to show that patients suffering from or just recovering from an attack of porphyria have a higher content of porphobilinogen in the urine than latent porphyrics. On the other hand it must be observed that the porphobilinogen content in some samples of urine may be high even in latent porphyrics.

This may lead to the question if there is any maximal excretion of porphobilinogen which is never surpassed. The problem is not easily solved as it is necessary to make the determinations on freshly voided urine, but the amounts excreted pro 10 kg body weight seem to agree remarkably well in the first group in which the patients have had recent subjective troubles (cf. the table 1) It may well be possible that there is an upper limit for the absolute amount of porphobilinogen excreted even if the concentration in the urine in a certain case with a heavy body weight and typical oliguria during an attack may be increased ten to twenty times during this stage of the disease. The high acidity of the urine favours the formation of porphyrin and porphobilin and this causes a very dark colour on standing and a consequent loss of porphobilinogen.

Different types of »acute porphyria».

The work on the heredity of the malady has given quite new possibilities to study the varying symptomatology of the disease. It is possible to trace the slightest symptoms of disease in the form of permanent or even transitory excretion of porphobilinogen in the urine. Such persons suffer from what Waldenström has named latent porphyria (20 of 103 cases in 1937). They were in my first publication suspected to be candidates for future severe porphyric attacks and this has later become true. Several of the persons, who were at first regarded simply as excretors of chromogen (porphobilinogen) have later developed such symptoms (Case 54 and the sister of Case 9 with chromogenuria), described in my monograph from 1937. Also some instances with negative findings in the urine in 1937 (a sister of Case 41, the father and a sister of Case 25) have later developed manifest porphyria.

Waldenström has pointed out, that there might exceptionally exist a condition called latent porphyria with intermittent porphobilinogenuria. This is a fact of considerable theoretical interest as many of the other inborn errors of metabolism have been explained as due to the lack of some normal enzyme necessary for the complete breakdown of that special product in the intermediate metabolism. Such an assumption could not possibly be true in acute porphyria as there exist cases with only transitory excretion of the pathological substance (porphobilinogen). In Waldenström's experience of about 150 different cases (several of these having been followed for up to nine years) this is a very rare condition. We have observed a complete disappearance of the porphobilinogen from the urine in altogether four cases. Some of these will be published later. This fact however seems to show, that there must exist all sorts of transitions between the fully developed porphobilinogenuria and the cases with only intermittent excretion. The table confirms this statement.

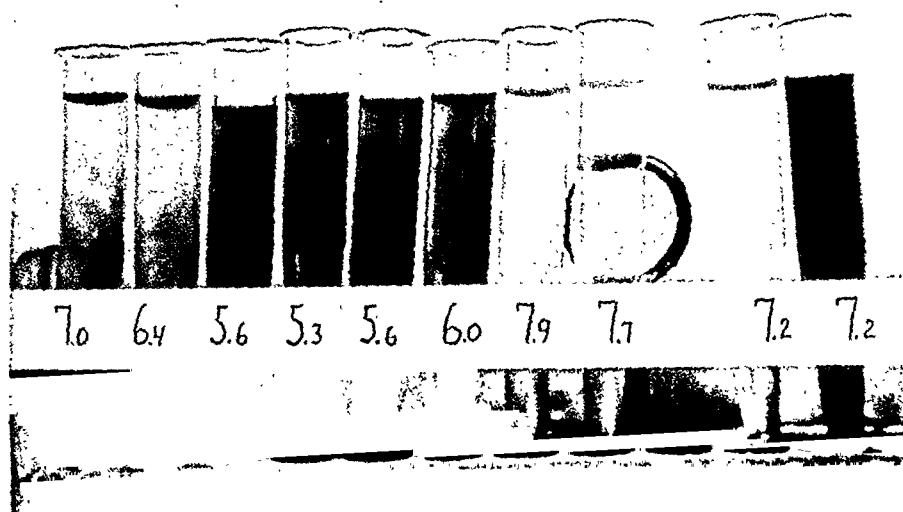
One of us (J. W.) has recently been able to observe 4 brothers belonging to a family of porphyrics. 2 of them have suffered from porphyric attacks with considerable excretion of porphyrin. At present they showed a very variable excretion of porphobilinogen. When the urine was boiled however it became reddish brown and it was possible to extract small amounts of uroporphyrin III, even when the porphobilinogen could not be identified with absolute certainty. In the two others no porphobilinogen or uroporphyrin could be identified. The possibility that the excretion of these substances may be intermittent has to be taken into account.

Another problem that has attracted considerable interest is the possible difference between the metabolic processes during the day and night. A very large number of determinations on Case I and II showed no definite differences of this kind as regards the excretion of porphobilinogen.

Influence of reaction on colour of urine.

The question about the efficacy of »alkali therapy» has received some attention since it was first introduced by Müller at the end of the last century. He found that the urine from a case of sulphonal porphyria had a very high acidity and therefore tried to give

The influence of P_H on the colour of the urine in acute porphyria



The first six tubes show the colour of the acid urine after standing. There has formed much uroporphyrin and porphobilinogen. When the urine is made alkaline through the administration of alkali to the patient there is a normal colour of the urine (tubes 7,9, 7,7 and 7,2). The last two tubes show the changes in colour when a urine with P_H 7,2 (left) is made acid and boiled for about 10 minutes (right). There are then momentarily formed the same large amounts of porphobilin and uroporphyrin as are seen when the acid urine is left standing at room temperature for some days or weeks. All the samples were 24 hour specimens and all contain practically the same amount of porphobilinogen.

the patient alkalis. The colour of the urine lightened rapidly and the condition of the patient improved after this treatment (and after the sulphonal medication had been stopped!). Our own experiments have shown quite distinctly that there is no change in the amount of excreted porphobilinogen. The light colour of the urine is therefore no indicator, that there is a real improvement in the metabolic disorder. On the other hand it does not seem improbable, that the neutralization of the tendency towards acidosis during the acute porphyric attack may have a favourable influence on the »toxic» condition.

Excretion of porphobilinogen and its relation to other clinical symptoms.

The mechanism of an acute porphyric attack with its varying symptoms from a great many organs might be explained in one of the two following ways. 1) It may be assumed that a faulty metabolism of some pyrrole compounds leads to the formation of (foreign) toxic substances. The malady would then have to be regarded as an autointoxication.

2) A rapid pathological breakdown of some necessary cell constituent containing pyrrole nuclei might result in a severe derangement in cell function. The cells are thus deprived of some necessary factor (enzyme?).

In both cases the excretion of porphobilinogen with the urine is the detectable sign of a disturbance in the synthesis or breakdown of pyrrole compounds.

Ad 1) In 1937 one of us (J. W.) put forwards the hypothesis that in acute porphyria there might exist a tendency to double carboxylation already of the simple pyrrole substances. Under such circumstances they were no more available for the synthesis of protoporphyrin and were therefore excreted. According to our view this theory might explain also the facts that have become known through our last chemical work. It has never been proved that porphobilinogen itself has any importance for the production of toxic symptoms. Other related substances might as well be made responsible. The experimental work by Waldenström and Wendt has shown that intravenous injections of porphobilinogen solutions

into rabbits do not cause any apparent toxic symptoms and the compound is excreted as such in the urine.

2) Another possible explanation would be that porphobilinogen is a break-down product from one of the enzymes of importance for the life of the cells. Schönheimer has shown, that the prosthetic group of myoglobin does not contain a uroporphyrin and hence the theory advanced by many authors about the connection between the uroporphyrin in acute porphyria and myoglobin, does not seem to be supported by the chemical facts. On the other hand the fact that the colourless porphobilinogen is the form in which the abnormal substance occurs inside the body makes it clear, that it is not necessarily derived from a coloured pyrrole substance. It may as well be present in the body in a non-coloured substance of essential importance for the cell-life at large (cf. the widespread disturbances from the alimentary tract, the kidneys, liver, muscles and central nervous system during a porphyric attack).

A thorough examination of urine specimens from a large number of normal persons always failed to show any porphobilinogen. It is thus evident that the occurrence of porphobilinogen even in minute amounts is a sure sign of porphyria. If subjective symptoms are absent the patient may be regarded as a latent porphyric, liable to get severe symptoms later on. In the families with porphyria I have also seen such cases with porphobilinogen in the urine who have never during a long life suffered from any symptoms that might be regarded as caused by a porphyric attack.

Summary.

1) The excretion of porphobilinogen in the urine has been followed quantitatively for a long period in two cases of acute porphyria.

2) When comparing six »active» cases with a recent porphyric attack with five typical cases of latent porphyria, it is evident that the porphobilinogen excretion is considerably higher in the former group than in the latter.

3) When reduced according to body weight the amount of porphobilinogen excreted is remarkably constant among the three cases with »active» porphyria.

4) A diet practically free from proteins did not alter the porphobilinogen output in case I. A severe reduction in protein intake was followed by no changes in case II. It is concluded, that porphobilinogen is probably an endogenous metabolic product.

5) The ingestion of alkali causes a rapid change in the colour of the urine but is not followed by any decrease in the excretion of porphobilinogen. The change in colour is only dependent on the rate of porphyrin and porphobilin formation from porphobilinogen in the urine. These changes are demonstrated on a coloured plate.

Zusammenfassung.

1. Die quantitative Ausscheidung von Porphobilinogen im Harn wurde längere Zeit hindurch bei zwei verschiedenen Kranken bestimmt.

2. Sechs Kranke, die neulich einen porphyrischen Anfall durchgemacht hatten, schieden bedeutend mehr Porphobilinogen aus als fünf latente Porphyriker.

3. Auf Körpergewicht umgerechnet ist die ausgeschiedene Porphobilinogenmenge auffallend konstant bei drei Fällen mit aktiver Porphyrie.

4. Starke Einschränkung der Eiweisszufuhr hat die Porphobilinogenausscheidung kaum beeinflusst. Es wird der Schluss gezogen, dass die Substanz hauptsächlich ein endogenes Stoffwechselprodukt sein dürfte.

5. Die Eingabe von Alkali bewirkt eine schnelle Änderung der Harnfarbe. Die Menge des ausgeschiedenen Porphobilinogens ist aber unverändert, und die hellere Farbe des alkalischen Harnes beruht nur auf die verhinderte Umwandlung des Porphobilinogens in Porphobilin und Porphyrin. Diese Tatsachen werden an einem Farbenphoto deutlich dargestellt.

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Reexamination of 79 X-ray-treated Exophthalmic Goiter Patients 8–18 Years after Their Discharge.¹

By

MOGENS BJØRNEBOE.

(Submitted for publication December 20, 1943.)

The increasing frequency of exophthalmic goiter, especially in the last year (Meulengracht)¹ has rendered the question of the medical treatment more topical. Nowadays probably most clinicians prefer subtotal thyroidectomy, which since the introduction of the Plummer iodine therapy may be said to be a rather safe operation. Still, cases occur continually in which special features of the disease in the given case of refusal of the patient to submit to operative treatment make it desirable to employ medical treatment with iodine or X-ray treatment. The question as to how much these methods of treatment may yield separately has not yet been elucidated fully. The present paper will deal only with the X-ray treatment.

As is well known, opinions are highly divergent as to the effectivity of this form of treatment. Presumably, however, it may be considered established that it is effective in several cases. Thus, by comparison of exophthalmic-goiter patients treated medicamentally and patients given X-ray treatment, Fenger (2) was able to demonstrate that recovery was obtained more rapidly with X-ray treatment. Means & Holmes (3) followed up their exophthal-

¹ Read before the Danish Society of Internal Medicine on May 30, 1943.

mic-goiter patients under X-ray treatment and showed that two-thirds of the patients improved in immediate connection with the treatment; and they claim that this outcome is not very likely to be accidental. Eggert Møller (4) found four-fifths of 29 patients improved in the first five months of the X-ray treatment, and he takes this to prove a causal connection between the X-ray treatment and the improvement.

The recovery percentage after X-ray treatment is given somewhat variably. In this country, Fischer (5) found recovery in 80 % of the cases, Fenger (6) in 50 % and Raagaard (7) in 78 %. Of reports from other countries it will suffice here to mention that Means & Holmes (8) give 67 %, Sanger (9) 82 %, Jaguttis (10), 76 %, Forsell (11) 75 %, Pfahler (12) 89 %, and Gantenberg (13) 59 % (these figures cover improved + recovered). However, only in a few of these works (Means & Holmes, Sanger) was the reexamination of the patients carried out after a suitable observation period, with personal examination on the patients and with determination of the metabolism. In several of the works, moreover, only a comparatively small part of the X-ray-treated patients could be summoned for reexamination.

I have tried to contribute to the question about the effectivity of this treatment by reexamination of the exophthalmic goiter patients under treatment in the Medical Dep. B of the Bispebjerg Hospital within the period of 1924—34. In these 11 years altogether 115 cases were treated in this department for hyperthyroidism. The criteria of the diagnosis were the classical complaints — emaciation, sensation of heat, profuse sweating, palpitation of the heart, nervousness, diarrhoea — and the physical findings: nervous restlessness, tremor, tachycardia, enlargement of the thyroid, exophthalmos, warm moist skin and — from 1928, regularly — measuring of the rate of metabolism. No distinction is made between exophthalmic goiter in the stricter sense of the term and toxic adenoma.

Of these 115 patients 22 were treated operatively; so they are excluded from the present material. In passing, however, it is to be mentioned that they represent no selection as the distribution of the cases between mild and severe among these 22 patients practically was the same as in the remainder of the material, although this group included no instance of arrhythmia perpetua. Of these

operated patients 5 died in immediate connection with the operation. Most of them were operated on before the introduction of the Plummer treatment. The remaining 93 patients were treated either with X-rays or medicamentally. In the autumn of 1942, *i. e.*, 8—18 years after their discharge from the hospital, I have tried to get in contact with these 93 patients. Information was obtained about 92 of them¹. Of these 92 patients, 17 have died, 69 were examined by me personally and 6 by means of a questionnaire. Determination of the rate of metabolism was performed on 54 of the personally examined, in most cases twice. Electrocardiography was employed in 52 cases.

Of the 92 patients, 7 were men, 85 women. The average age was 36 years (varying from 10 to 64). The classification of the cases after the degree of illness is carried out after Sällström (14) who merely distinguishes between severe and mild cases, setting the borderline at a metabolic rate of 140—150 %, and a pulse rate of 100—110, and with the loss of weight and general condition taken into consideration. The severe cases are again divided into cases with and without exophthalmos. The present material contained the same numbers of severe and mild cases. Of the 46 severe cases, 27 had exophthalmos. The severe cases without exophthalmos include the so-called toxic adenoma, which gives a higher average age (41 years) for this group. Of the total 92 patients 10 had arrhythmia perpetua.

The treatment of the 92 patients was entirely medicamental in 13 cases (stimulants, sedatives and iodine). In 79 cases X-ray treatment was given.

The 13 patients receiving only medicamental treatment are to be mentioned but briefly. They represent a selection insofar as they include the very severe cases in which it was not found safe even to employ X-ray treatment. Four of these patients died in the hospital or shortly after their discharge; one died later. (Two of these patients died of cardiac insufficiency, one of thyrotoxic crisis, one of diabetic coma and pneumonia, and one of pulmonary embolism post partum.) Of the surviving 8, four are well, three are suffering from cardiac insufficiency, and one from epilepsy.

¹ The one patient who could not be located was a woman, aged 32, with the degree of illness +. She was treated only with iodine.

² — *Acta med. scandinav.* Vol. CXVII.

The 79 X-ray treated patients represent the more important part of the material, including 38 severe and 41 mild cases. Of these 79 patients eight were later given operative treatment, 2—7 years (on an average 3.3 years) after their discharge; they were all more or less ill in the interval between their discharge from Dep. B and the operation. Of these 8 patients, four are now well, while four are ill. Of the latter, 1 is suffering from myxoedema and 1 from cardiac insufficiency; concerning the remaining two, information has been obtainable only by mail: one suffers possibly from myxoedema, the other from a relapse of hyperthyroidism.

This leaves 71 patients who were given X-ray treatment alone. Of this total 12 died, 59 are living. The causes of death in the 12 cases are given in Table 1. The death occurred from 0 to 18 years

Table 1.

X-ray-treated, later dead patients (12).

Thyrotoxic crisis.....	2
Cardiac insufficiency	4
Hyperthyroidism, Diabetes	1
Cancer ventriculi	2
Meningomyelitis	1
Meningitis.....	1
Pyelonephritis sin.....	1

after the discharge of the patient, on an average 5 years after. The average age at the death of the patient was 49 years. Autopsy was performed on both the patients who died of thyrotoxic crisis. Autopsy was also performed on one of the patients who died of cardiac insufficiency, revealing mitral stenosis. No detailed information could be obtained about the patient who died of hyperthyroidism and diabetes. From Table 1 it will be noticed that hyperthyroidism probably was a contributory cause of death in 7 of the 12 cases (the first 3 groups in the table).

Of the 59 patients who were living at the time of the reexamination, 44 were well, 15 ill.¹ Of these patients 50 were reexamined with determination of the metabolism. On their admission to the hospital, most of the 44 recovered patients (two-thirds of them)

¹ One of the 45 «recovered» mentioned in the address 30. 5. 43. who refused to submit to determination of the metabolism was found on a subsequent examination to have a metabolism of 79—82 %.

presented mild cases of hyperthyroidism, while the entire material (the 71 X-ray-treated, non-operated patients) included the same numbers of mild and severe cases. Thus the recovered patients belong chiefly to the group of mild cases. Several of the recovered patients presented some remaining symptoms. 34 % showed exophthalmos (as against 68 % on admission) and 7 % showed enlargement of the thyroid (as against 84 % on admission). One-third of the recovered have to be characterized as »nervous» persons. The duration of illness after the institution of treatment is evident from Table 2. The duration of illness is reckoned from the admission to the point of time when the patient felt well and was able to resume the usual work to full extent. It will be noticed that 77 % recovered in two years or less.

Table 2.

X-ray-treated, at the reexamination cured patients. Duration of illness after admission.

0—½ year	16	} 34 = 77 %
½—1 "	11	
1—2 years	7	
2—3 "	3	
3—4 "	2	
4—5 "	1	
5—6 "	1	
6—7 "	3	

At the reexamination 15 of the X-ray-treated patients were ill. The causes of the illness are given in Table 3. The 8 cases of hyperthyroidism were all relapses. In 6 of these cases the free interval between recovery and relapse was several — up to 12 — years. In 5 of these cases the relapse occurred in 1941—42 (in one instance, in response to a reducing cure with thyroidin). Of the eight cases of relapse, four were severe, four mild (including one doubtful). The myxoedematous patient showed a metabolic rate of 80 % and was also suffering from arthrosis involving several joints. All the three patients with cardiac insufficiency presented arrhythmia perpetua; one of them was suffering from the sequelae of apoplexy with hemiplegia. None of them presented characteristic murmurs on auscultation.

In the X-ray Dep. of the Bispebjerg Hospital, in the period of 1924—34, the X-ray treatment for hyperthyroidism was given after two fundamentally different methods: 1) irradiation of one field — the anterior surface of the neck — and 2) radiation in 3 different directions (from the right, from the left, and anteriorly) and these three radiations are given at intervals of a few days.

Considering the entire period of 1924—34, irradiation of a single field was found to be the dominating method prior to 1928, whereas employment of three different directions of the rays dominated after 1928. As to the individual dose, it has varied between $\frac{1}{2}$ and 2 Sabouraud and 150—450 r. In the later years the dosage was controlled with the Baastrup-Johnsen dosimeter. The filter was 4—5 mm aluminium. The distance employed was 23 cm in 1924—28, 33 cm in 1929—31, and 40 cm in 1931—34. The treatment was repeated at intervals of one or more months, depending on the clinical symptoms and rate of metabolism. The treatment was individualized with regard to the size of the single dose as well as to the frequency of the radiations. Thus the duration of treatment varied from a single radiation or series of radiations to treatment through seven years. Thus one patient was given 1—5 series of treatment a year in 1924, 25, 28, 31 and 32. On an average the patients received 5 irradiations of a single field or 3 serial irradiations with 3 different directions of rays.

The duration of the treatment for the 44 recovered patients is recorded in Table 4. The distribution of the duration of treatment for the ill patients and for those who died shows no distinct difference from the distribution in the group of recovered. Further, no difference can be demonstrated in the results of the treatment for the patients treated before and after 1928, when the method of treatment was altered.

Table 3.

X-ray-treated, at the reexamination ill patients (15).

Hyperthyroidism	8
Myxoedema	1
Cardiac insufficiency	3
Diabetes mellitus	1
Morbus Meniere	1
Neurasthenia	1

Table 4.

Duration of X-ray treatment for 44 recovered patients.

0— $\frac{1}{2}$ year	26	} 38 = 86 %
$\frac{1}{2}$ —1 »	5	
1—2 years	7	
2—3 »	3	
3—4 »	1	
6 »	1	
7 »	1	

Table 5.

Result of X-ray treatment.

All X-ray-treated cases minus patients, who have died (5) and are ill (3) from affections without connection with hyperthyroidism: 71.

Of these 71 are		
Dead	7	(9.9 %)
Sick	12	(16.9 %)
Later treated operatively	8	(11.2 %)
Cured	44	(62.0 %)

A comparison between the doses here employed and the doses employed in the works cited above is impracticable because the authors have failed to give sufficient information about details in the technique of irradiation. Here it is to be emphasized that no inconvenience from the X-ray treatment was observed in any case in the form of any skin lesion. The only patient with severe changes in the skin had been treated previously in the earliest days of X-ray treatment.

As to the risk of myxoedema, two cases of this kind are found in the present material. One of these patients underwent thyroidectomy after the X-ray treatment; the other received only X-ray treatment. The latter patient received two series of X-ray treatment in 1924, respectively 2 and 1 $\frac{1}{2}$ Sab. \times 3, with an interval of two months. Arrhythmia perpetua did not arise in any case after this treatment; wherever this condition was found, it was present also during the patient's stay in the hospital.

The total result of the reexamination is evident from Table 5. It will be noticed that about two-thirds of the patients recovered under this treatment. This outcome is quite in keeping with the

findings reported by other investigators, especially Means & Holmes, whose work has to be reckoned among the very best in this field. If we compare the results obtained with X-ray treatment with the results from operative treatment — *e. g.*, in the comprehensive account of Windfeld (1940): percentage of recovery 90, and operative mortality 2.8 % — we have to admit the superiority of the operative methods, not least when we consider how quickly the result is obtained with this method (97.6 % recovered in less than one year).

The argument that X-ray treatment has no case mortality holds good only with certain reservations. For, during the protracted treatment, the patients are apt to die from thyrotoxic crisis or from their heart lesion which might have been cured by the operation. Still, presumably X-ray treatment will remain employable in certain cases — namely the very severe and patients who refuse to submit to operative treatment.

Summary.

A total of 79 exophthalmic goiter patients who were given X-ray treatment are followed up 8—18 years after their discharge. Information is obtained about all of them. At the reexamination now, the rate of metabolism was determined on most of the patients still alive.

Of these 79 patients 5 died and 3 are suffering from affections irrelevant in this connection. Of the remaining 71 patients 8 were later treated operatively (11.2 %), while 7 died (9.9 %), 12 are ill (16.9 %), and 44 are well (62.0 %).

In 2 cases the cause of death was thyrotoxic crisis, in 4 cases it was cardiac insufficiency. Of the sick patients 3 suffered from cardiac insufficiency, 8 had a relapse of hyperthyroidism, and 1 had myxoedema.

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Some Remarks on The Variation with Latitude of The Steepness of The Epidemic Curves of Poliomyelitis.

By

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(Submitted for publication October 15, 1943).

In Acta medica scand. 1943: 115, 560, after making a summary of the epidemic theory laid down by me, Herman Wold has given an account of an attempt, made by him, to test the regular variations with latitude of the steepness (or the dispersion) of the epidemic curves of poliomyelitis on the basis of some Swedish epidemics. Herman Wold finds that his test does not confirm my results and states that my theory «contrasts keenly to other approaches».

I would like to take the opportunity of making a few remarks in connection with this account.

Considering the epidemic curves of poliomyelitis as normal curves of distribution, and computing the dispersion, μ , for a number of epidemics from various countries, I have found (see my papers in Acta medica scand., fasc. III—V 1941 or Nordisk Medicin 1942: 16: 3509) that, though the value of dispersion for the individual epidemic may exhibit deviations, these deviations are quite independent of *the extent of the epidemics* (which in respect of epidemics in New York ranges from 100 to 13000 cases) as well as of *the size of the population* (which as regards Danish epidemics varies from 40,000 to 1 million).

Further, the deviations of the individual epidemics in the individual country are in most cases so small that the *mean values* of μ for the individual country may be determined with a mean error varying by 2—10 per cent, whereas the μ -values for the different countries vary by up to 200 per cent, in such a manner that μ increases quite regularly, when going from countries in high latitudes to countries in low latitudes. It thus appears that the value of μ yields an adequate quantity for expressing numerically the general experience that epidemic curves of poliomyelitis are steeper in high latitudes and smoother in low latitudes.

I wish to emphasize in this paper, as I have already done in «*Nordisk Medicin*» (p. 3520), that the said regularity only appears distinctly for *small* latitude variations, when *mean values* of μ for several epidemics from the same latitude are available, owing to the fact that the individual epidemics may occasionally exhibit rather considerable deviations. Now Herman Wold uses only one epidemic from one particular locality, and therefore it is by no means surprising that he does not find any regularity. However, computing the mean value of the 17 epidemics, used by Herman Wold, we get $\mu = 3.7 \pm 0.2$, and computing the «gravity centre» of the corresponding latitudes, we get about 59° ; and according to fig. 13 in my paper in *Nordisk Medicin* 1942: 16: 3509 the value of μ_{59} should be 3.4. This accordance must, I think, be called rather good. — Examining the individual epidemics in Wold's graph, we see that it is only the three epidemics at 56° and the two epidemics at 63° , which deviate to any essential degree. Actually, computing *mean values* for $\mu_{57.5}$ (2 epidemics), $\mu_{58.5}$ (4 epidemics), and $\mu_{59.5}$ (6 epidemics), we get the values 4.2, 3.8, and 3.5; obviously, these values confirm very nicely the «normal» decrease of μ with increasing latitude, though the values being a little too high. — But it must be admitted that the values for 56° and 63° exhibit rather great deviations.

However, as already stated in my previous paper, I might add several more examples of epidemics, which do not agree with the regularity in question. When going through the tables in Wernstedt's great treatise of the epidemics in Sweden during the years 1911—1913, it is found that most of the local epidemics, on account of their irregular course, do not permit a computation of μ ; nor do the epidemics, which appear to have a regular course, with a

few exceptions, give a value of μ , which agrees with the general rule.

Now, the irregularity which occurs in minor epidemics of poliomyelitis may to a certain degree be accounted for by the difficulty in establishing the *correct time* for the setting in of the single case; for, obviously, seeking the form of the epidemic curve, i. e. the variation with time of the occurring number of cases, it must be of importance that all cases are »picked up» at the same stage of the development of the disease; if some of the cases are noted at the time of the first sign of the illness and others on the day of hospitalizing, deformations of the curve may occur; this source of error is of course without any connection with the correctness of the diagnosis, but the errors may occasionally be of systematic character and thus give rise to irregularities. But it must also be borne in mind *that the very assumption that the matter of infection be »ubiquitous», involves the possibility of great and characteristic deviations from the regular course of the epidemics*; for, as emphasized in my paper in *Nordisk Medicin* (p. 3522), the appearance of the regular theoretical course of the epidemic depends on the presence of a *stationary state* of the ubiquity of the matter of infection, and every alteration of this state during a running epidemic must entail a corresponding deviation from a regular course of the epidemic.

Dealing with Swedish epidemics, it is pertinent in this place to mention as an example the theory of C. Kling. This theory maintains that waters are of essential importance for the dispersion of the matter of infection on account of their containing tiny organisms, which may act as intermediary hosts for the matter of infection. If this theory proves right, we have got *one* of the different ways by which the ubiquity is realised. (In connection herewith I would like to point out that the theory of ubiquity thus *embraces* the results of C. Kling rather than »contrasts» to them.) Other ways, in which the ubiquity may be realised, are possibly more directly connected with the sick individuals, although normally, they need not consist in an actual transmission of a matter of infection from one case to the next new case. Finally, and this possibility should not be overlooked, also »sound carriers» may be active in establishing the said ubiquity, besides which there may still be other ways altogether different from those above mentioned. The stationary state involves that *none* of these different mecha-

nisms, contributing towards spreading the matter of infection, are prevailing, and only *then* should the regular curve be considered the most probable one. But if the different mechanisms have not reached their full height of intensity, one — or several — of them may prevail, and, this being the case, the course of the epidemic will undoubtedly be correspondingly affected by the prevailing mechanism, and the shape of the epidemic curve will consequently not exhibit the characteristics of the curve representing the variation of the resistance of the individuals, as assumed in type c, nor the variation of the virulence of the matter of infection. (Statistics as such cannot distinguish between these two complementary possibilities). This state of «imperfect ubiquity» is most likely found in regions with many waters (if Kling's theory be right), sparse population and little traffic; consequently it does not seem strange that sparsely inhabited counties as Västernorrland and Jämtland do exhibit deviations.

In part 7 of his paper Herman Wold makes the conjecture that the «latitude relation», mentioned in my papers, be without any reality, on the assumption that the found variation of μ is a result of some unknown effect of variations in the size of the population, which is in direct opposition to the *independence* of the size of population, found by me (see introduction). — Yes, this *may* of course be so. The case *may* also be that the found variation of μ with latitude is the mere result of various casual irregularities of the above named nature, — and, by the bye, there is also the possibility that the relation, claimed by C. Kling, between the dispersion of the cases of poliomyelitis and the presence of waters, is to be attributed to a partiality in the population of settling down in regions with waters, and not to the effect of possible intermediary hosts (see Birger Jönsson: Zur Epidemiologie der Kinderlähmung, Stockholm 1938). As far as the author has grasped the matter, several features of the epidemiology of poliomyelitis do not agree with this theory.

On the whole it stands to reason that if we allow ourselves to be satisfied with pure *conjectures*, we may find numerous different «explanations». But that seems to me to be rather unsatisfactory. If in want of an explanation of the latitude relation, you *may*, as Herman Wold apparently suggests, accept a mere conjecture about *potential* causes. But you may also try to penetrate deeper

into the matter and find an agent by which to explain the relation to latitude. Obviously such a task must, at the beginning, be an attempt only; but the establishment of most »laws«, governing the phenomena of nature, have undoubtedly developed from such attempts, and I find it permissible to state that only continued *numerical* investigations, and not mere conjectures, can weaken or confirm the *numerical* evidence given by me for the existence of the relation between the dispersion of the epidemic curve of poliomyelitis and the variation of light with latitude.

Finally I wish to add that I agree with Professor Wold in his opinion that my poliomyelitis theory rests on a weaker basis as compared with the theories a and b (Wold's notations); for — fortunately — poliomyelitis is a rare disease, so that it has not been possible to perform the test in respect of this disease to the same extent as in respect of the common diseases; and this, most especially, holds good with regard to the supposed relation to light. It should only be taken as a first attempt to detect a trace of lawfulness in a complex of phenomena, which, so far, have been rather puzzling. On the other hand, it seems rather difficult to regard the relation found as a purely haphazard phenomenon, as Professor Wold proposes, when so many mutually independent features fit in so astonishingly well.

If, from a *biological point of view*, a relation to *light* is to be considered less probable (I am no biologist), the correct way should in my opinion be to find another cause for the well known relation to latitude, which fits in satisfactorily with the observed behaviour of a great number of epidemics.

(From the Biological Laboratory of «Medicinalco» Ltd. Copenhagen).

Investigations on the Content of Reticulocyte-Ripening Substances in the Plasma in Various Forms of Experimental Anemia.

By

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(Submitted for publication December 13, 1943).

In reports of previous investigations an account was given of the content of reticulocyte-ripening substances in plasma derived from a number of different animal species [C. M. Plum 1943 (3)]. Here it was established that there is a certain correlation between the content of ripening substances in the plasma and the amount of reticulocytes in the circulating blood, animal species in which normal full-grown animals have numerous reticulocytes showing a corresponding smaller amount of ripening substance than animals with few reticulocytes. In a number of cases in which erythropoiesis was increased, an increased amount of ripening substances was demonstrated in the plasma. This applies for instance to young, not hematologically full-grown, animals, and to animals made anemic by repeated blood-letting [C. M. Plum 1943 (4)].

On the other hand, we have ascertained that the plasma of patients suffering from pernicious anemia (a single case reported by Jørgensen & Plum, 1943) contains less ripening substance than the plasma of normal human subjects [C. M. Plum 1942 (2)].

In order to elucidate the connection between the content of ripening-substances in the plasma and the erythropoiesis it is, therefore, of interest to ascertain the behaviour of the ripening

substances in different forms of anemia. So in the present paper I have examined divers forms of experimental anemia and their influence on the content of both fully ripe and preformed (i. e. tyrosine-activable) ripening substance in the plasma.

Most forms of anemia may be classed in one of the following three groups:

1) A deficient supply of raw materials such as iron, copper etc, that is to say, substances co-operating in the formation of hemoglobin. These are the so-called *deficiency anemias*.

2) Increased necrosis of erythrocytes, the organism not being able to satisfy the consumption by its own aid. These are the so-called *destructive anemias*, and

3) a deficient function of the bone marrow, that is to say, the *hypo- or aplastic anemias*.

[See also W. B. Castle & G. R. Minot, Pathological Physiology and Clinical Description of the Anemias (1936)].

Milk Anemia.

Scott (1923) reported that the young of rats fed exclusively on milk became markedly anemic while the mothers did not develop anemia. Hart, Elvehjem and co-workers (1928) showed that if young rats are put on a diet consisting exclusively of milk they develop anemia. If rats in which anemia had thus been induced through the *one-sided diet* were given iron, the iron supply of the organism increased but no effect could be traced on the hemoglobin formation; but if in addition to the iron they were also given a supply of copper a rise would be noted in the amount of hemoglobin, as a rule accompanied by a reticulocyte crisis.

The form of anemia developing on a milk diet thus seems to be due to a deficiency of iron and copper in the food.

Method. For the experiments with milk anemia in this laboratory were employed male as well as female white rats one month old and weighing 40—70 g (average weight 55 g).

Four male and four female rats were placed in glass containers: The floors of the glass containers were covered with shavings and sawdust, the lids were of wood so that the animals did not come in contact with metal in the experimental period. Four male rats, representing the controls, were placed in ordinary wire cages.

Diet. The rats in the glass containers received whole milk (stassanised) ad lib. while the four controls received the breeding diet generally employed in this laboratory consisting of

skim milk powder	15	kg
potato flour	20	kg
dry yeast	3.75	kg
arrachis-oil	7.50	kg

+ 3000 units of vitamine A + 100 units of vitamine D per kg mixed food.

Analyses of the blood.

All the investigations were made on blood derived from the caudal veins. The blood was taken as previously described [C. M. Plum 1943 (5)], first for the usual analyses (for hemoglobin, erythrocyte-leucocyte-reticulocyte counts, and smears for the differential count) in a dry test tube with dry oxalate-fluoride at the bottom (9 g potassium oxalate + 1 g sodium fluoride), after which the amount of blood required for determination of the ripening index in the ripening experiments [for the method see C. M. Plum 1942 (1)] was collected in another test tube, coagulation being prevented by sodium citrate (3.8 % solution). The results will appear from Tables 1 and 2.

In all the rats on the milk diet a fall was seen in the amount of hemoglobin, even though it cannot by any means compare with the results shown by Hart & Elvehjem who found that the amount of hemoglobin was reduced to 33 %, while in the present experiment only a fall to 60 % was found. Some of the animals, simultaneously with the fall in the hemoglobin formation, showed a rise in erythrocytes. The diameter of the erythrocytes in these cases is reduced by 15—25 %. Hence a microcytotic hypochromous anemia has developed. Coincident with the onset of the anemia the organism itself tries to improve conditions by an increased erythropoiesis, as evidenced by the increased amount of reticulocytes.

The reason why we did not find as great a fall in the amount of hemoglobin as other workers, may possibly be that we used stassanised milk for the investigations, which unlike the raw milk of earlier investigations has been exposed to close contact with large copper plates.

Table 1. Analysis of the blood of 4 rats on a milk diet with subsequent

Diet	16/4—12/6 Milk				16/4—12/6 Milk				16/4—12/6 Milk		
Sex	♂				♂				♀		
Date	16/4	15/5	12/6	28/6	16/4	15/5	12/6	28/6	16/4	15/5	12/6
Weight g	63	134	182	204	46	104	158	168	70	127	154
Hemoglobin %	100	81	7	116	94	90	79	109	100	84	74
Erythrocytes ..	6.83	9.01	7.23	8.51	7.90	10.17	7.21	8.39	8.92	8.73	10.03
Leucocytes	9.240	7.200	7.640	9.840	8.680	8.240	7.440	8.620	8.420	6.580	8.120
Reticulocytes ..	11	60	106	10	16	72	86	12	12	83	142
Ripening index	0.83	0.87	0.94	0.80	0.79	0.88	0.99	0.79	0.80	0.89	0.99

Table 2. Analysis of the blood of 4 rats on a milk diet with subsequent

Diet	16/4—29/5 Milk				16/4—29/5 Milk				16/4—29/5 Milk		
Sex	♂				♂				♀		
Date	16/4	15/5	29/5	18/6	16/4	15/5	29/5	18/6	16/4	15/5	29/5
Weight g	77	155	163	245	47	112	123	206	40	88	90
Hemoglobin %	100	82	74	103	105	84	70	94	96	96	74
Erythrocytes ..	7.93	8.53	11.51	5.89	8.31	9.23	10.86	6.42	8.29	9.80	12.24
Leucocytes	7.440	9.840	8.120	8.440	8.440	8.200	9.240	11.860	7.660	10.200	9.320
Reticulocytes %	12	85	136	16	10	70	152	10	10	66	160
Ripening index	0.79	0.91	0.96	0.78	0.80	0.90	0.98	0.80	0.80	0.84	0.98

Upon a change of diet, either after giving the animals an additional amount of iron or copper (Table 1) or by substituting for the milk diet the ordinary breeding diet, the animals' requirement of iron is covered, and after a while normal conditions will again be seen in the blood picture.

Whether the effect of this supply of iron is exclusively of importance for the formation of hemoglobin it is difficult to say; the animals on the milk diet did not thrive well (cf. the weight curve), but with the addition of iron a distinct increase in weight

addition of iron and copper plus 2 rats on the ordinary breeding diet.

12/6—28/6 Milk 0.5 mg FeCl ₃ 0.25mg CuSO ₄	16/4—12/6 Milk				12/6—28/6 Milk 0.5 mg FeCl ₃ 0.25 mg CuSO ₄	16/4—28/6 Breeding diet				16/4—28/6 Breeding diet			
	♀					♂				♂			
	♀					♂				♂			
28/6!	16/4	15/5	12/6	28/6	16/4	15/5	12/6	28/6	16/4	15/5	12/6	28/6	
167	41	99	129	156	63	164	263	290	40	139	212	235	
129	115	81	66	124	96	95	99	113	104	100	112	106	
8.38	8.55	8.47	9.53	9.75	8.21	9.81	8.90	9.94	8.67	8.55	7.86	8.96	
10.220	8.420	9.940	7.240	8.960	7.640	8.600	10.640	7.460	6.480	8.920	10.240	9.260	
16	10	82	127	15	10	20	13	12	10	10	16	16	
0.79	0.82	0.86	0.96	0.83	0.79	0.79	0.80	0.80	0.79	0.79	0.77	0.78	

change to breeding diet, plus 2 rats on the ordinary breeding diet.

29/5—18/6 Breeding diet	16/4—29/5 Milk				29/5—18/6 Breeding diet	16/4—18/6 Breeding diet				16/4—18/6 Breeding diet			
	♀					♂				♂			
	18/6	16/4	15/5	29/5		18/6	16/4	15/5	29/5	18/6	16/4	15/5	29/5
148	67	139	150	202	62	131	174	233	48	154	204	251	
96	112	82	72	95	109	99	98	102	96	96	96	111	
7.67	9.32	9.25	12.12	9.33	9.51	8.98	7.90	8.66	9.67	10.80	8.12	7.06	
8.820	7.460	8.060	7.840	7.340	9.460	6.200	8.680	9.820	8.260	8.120	7.680	8.480	
11	11	56	187	14	10	10	13	13	8	10	11	6	
0.82	0.81	0.85	0.96	0.80	0.82	0.82	0.82	0.83	0.82	0.82	0.80	0.81	

was seen, so that it must be concluded that the iron not only affects the formation of hemoglobin but also some of the other functions of the organism.

Conclusion.

In animals where the supply of iron is reduced as a result of a diet without iron a microcytotic hypochromic anemia is seen to develop. The anemia is accompanied by reticulocytosis and an increased formation of reticulocyte-ripening substances.

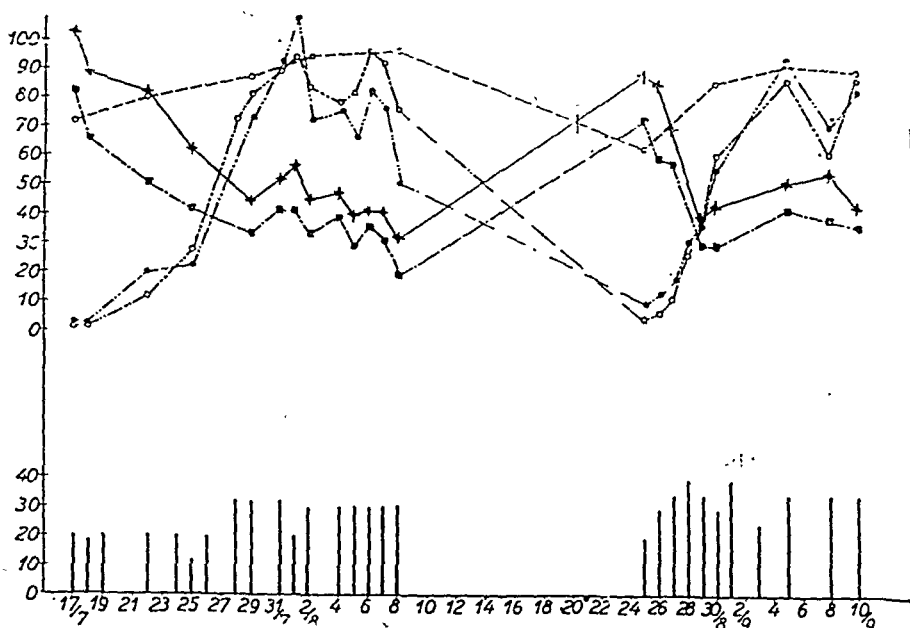


Fig. 1. Variations in the blood picture and in the ripening index during two periods of chronic hemorrhagic anemia between which the animal attained an almost complete remission.

Abscissa: Time in days.
 Ordinate: ■ — ■ Percentage of hemoglobin (Zeiss Ikon).
 (100 = 100 %).
 × — × No. of erythrocytes in millions in mm³.
 (100 = 5 mill.).
 ○ — ○ No. of reticulocytes per thousands.
 (100 = 200).
 ● — ● No. of reticulocytes in mm³ (calculated from erythrocytes).
 (100 = 500,000).
 ○ — ○ Reticulocyte ripening index.
 (100 = 1.00).
 ■ Quantity of blood taken in cm³.

Hemorrhagic Anemia.

For these experiments white male rabbits were used. In the experimental period they were kept in separate cages on a diet consisting of hay and cabbage-leaves.

The animals were rendered anemic by heart puncture daily or every other day, about 40 cm³ of blood being taken each time.

On inducing anemia a rapid rise is observed in the number of reticulocytes until a plateau is reached round which the figures fluctuate. The percentage of hemoglobin and the number of

erythrocytes fall and form a plateau (see the figure). After a period of long treatment a premortal fall in the number of erythrocytes and the percentage of hemoglobin is often seen, accompanied by great fluctuation in the number of reticulocytes.

If the heart punctures are stopped a further rise in the number of reticulocytes is often observed the next day, after which a fall occurs accompanied by a rise in both the hemoglobin percentage and in the number of erythrocytes, and in the course of 2—3 weeks the animal is practically restored, Schwarz, 1940. As regards the ripening index, it will be seen from Fig. 1 that this rises. The ripening index rises to a plateau that appears simultaneously with the rise in the number of reticulocytes and falls to normal when the blood-letting ceases in the same time as is necessary for the animal to be restored.

Conclusion:

From these experiments it appears that the concentration of ripening substances in plasma rises when anemia is induced.

It seems evident that the rise in the concentration of ripening substances can be regarded as a compensatory measure on the part of the organism for the loss of blood, like the compensatory hypertrophy of the marrow during chronic hemorrhagic anemia.

Hydroxylamine Poisoning.

Hydroxylamine is a blood poison which converts hemoglobin into methemoglobin, like for instance phenylhydrazin, phenylhydroxylamine and hydrazin.

If hydroxylamine is given in large amounts to the experimental animals a vigorous destruction of blood sets in which will cause death. If, on the other hand, small doses are administered daily the hydroxylamine, like other related substances, will at first cause a severe anemia which will later subside somewhat. This is partly because the erythrocytes become more resistant than normal (Morawitz & Pratt, 1908, Suzuki 1912), but in the first place because the erythropoietic system increases its activity (Tallqvist, 1899, Duesberg, 1931).

In this treatment with hydroxylamine a regenerative anemia is observed, i. e. a form of anemia which is characterised by an

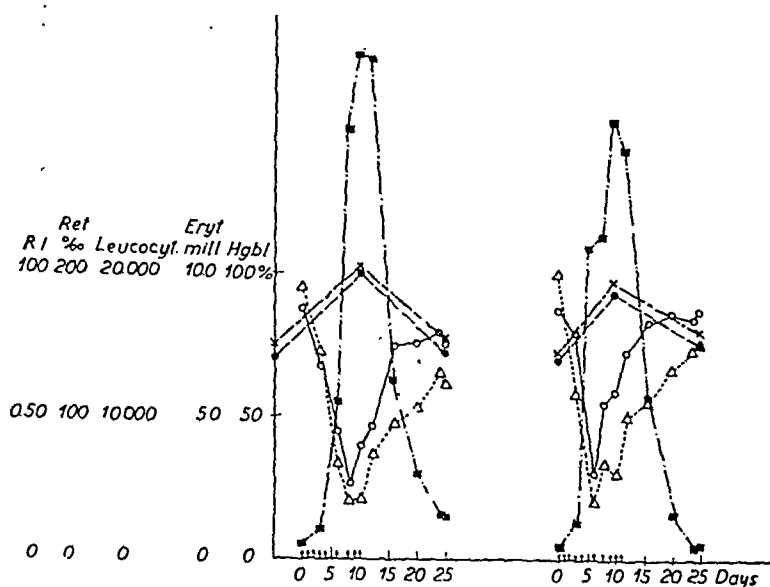


Fig. 2. Variations in the blood picture and ripening index during hydroxylamine poisoning.

- Reticulocytes in %.
- △—△ No. of erythrocytes in millions in cm^3 .
- Percentage of hemoglobin (Zeiss Ikon).
- Ripening index for plasma.
- ×—× Ripening index for plasma plus tyrosine ($0.1 \text{ mm}^3 1\%$).
- ↑ ↑ ↑ Injection of hydroxylamine.

enormous activity of the bone marrow which is taxed to the utmost to check the destruction of the blood, (see further Jacobsen, Plum and Milwertz, 1939).

The experiments were performed on white male rabbits kept in separate cages during the experimental period, on a daily diet of hay and cabbage leaves.

Daily examinations of the blood were made on venous blood from the ear: the determinations were: hemoglobin determinations, counting of erythrocytes, leucocytes and reticulocytes. At intervals the content of ripening substances in the plasma was determined by the method previously described [C. M. Plum 1942 (1)], the blood for these investigations being taken ad modum Sjöwall (1936). The animals received daily an intravenous injection in the ear of an aqueous solution of hydroxylamine hydrochloride, 0.5 cm^3 corresponding to 5 mg of hydroxylamine hydrochloride. They were given 0.5 cm^3 per kg body weight. The solution was

neutralised to pH 7.2, too acid solutions causing severe pain and thrombosed veins. The rabbits tolerated these injections well.

The results will appear from Fig. 2.

As will be seen, a fall very rapidly occurs in the hemoglobin percentage and the amount of erythrocytes, accompanied by a rise in the number of reticulocytes.

On examining the content of reticulocyte-ripening substances in the plasma a rise in these was found, corresponding to the rise in the reticulocytes. This must be interpreted as a compensatory production of ripening substances to prevent the great fall in erythrocytes, exactly like that observed in the animals with hemorrhagic anemia.

When the injections were discontinued a gradual increase was seen in the erythrocytes, accompanied by a rise in the hemoglobin percentage and a fall in the reticulocytes. At the same time a fall was observed in the amount of ripening substances.

Conclusion.

Treatment with small doses of hydroxylamine is seen to cause anemia accompanied by a rise in reticulocytes and in the amount of reticulocyte-ripening substances in the plasma. This must be regarded as a compensatory process on the part of the organism in order to make up for the increased destruction of the blood.

Benzol Poisoning Anemia.

Chronic benzol poisoning is generally stated to be accompanied by a reduction in the function of the bone marrow.

The benzol tolerance, however, varies individually.

The effects of benzol poisoning manifest themselves especially in the change in the blood and in injuries to the bone marrow. In individuals who are particularly sensitive to benzol pronounced leucocytopenia and thrombopenia are seen: the bone marrow becomes hypo- or aplastic, and distinct necrobiotic changes appear in the granulocytes. In individuals less sensitive to benzol few changes occur in the blood picture, the function of the bone marrow is as a rule unaltered or a slight hyperplasia may be seen. Further particulars may be gathered from »Pathological Physiology and

Clinical Description of the Anemias» (W. B. Castle & G. R. Minot, 1936); Handbuch der experimentellen Pharmakologie, 1 Bd. 1923; and Downey's Handbook of Haematology, 1938.

Method.

White male rabbits were used (weight 2250—2800 g). During the experiments they were kept in separate cages on a diet of hay and cabbage leaves.

Daily determinations were made of the hemoglobin percentage, and the erythrocyte, leucocyte and reticulocyte count on blood taken from an ear vein. At intervals 5 cm³ of blood were taken from an ear vein ad modum Sjöwall (1936) to determine the content of ripening substances in the plasma.

The benzol poisoning was carried out in the following way. 2.5 cm³ of benzol plus 2.5 cm³ of arrachis-oil were injected subcutaneously every day in the back, a method indicated by Schilowa amongst others (1930).

The results of two experiments will appear from Fig. 3.

These two animals died in the course of 6—8 days, one immediately after an injection. The other was killed, as a total paralysis of the hindpart following a fracture of the column set in. Two other animals were likewise treated with benzol injections subcutaneously, but as there was not the least trace of a response after 10 days the injections were discontinued.

The method suggested by Langlois & Desbouis (1907) for experimental benzol poisoning was tried on a number of rabbits, 2 cm³ of benzol being injected intravenously into rabbits weighing 2500 g, but the animals died immediately after or during the injection. Smaller doses were tried but even doses of 0.2 cm³ caused the death of the animals.

Some few rabbits were treated with subcutaneous injections for some time but when a suitable fall in the amount of erythrocytes, hemoglobin and leucocytes had set in, the injections were discontinued (Fig. 4).

As will be seen, in some of the cases there is a tendency to leucocytosis at the beginning of the experiments, as described by Schilowa (1930). But after this a fall occurs in the amount of the leucocytes. The number of erythrocytes decreases during the injections on a line with the amount of hemoglobin. The reticu-

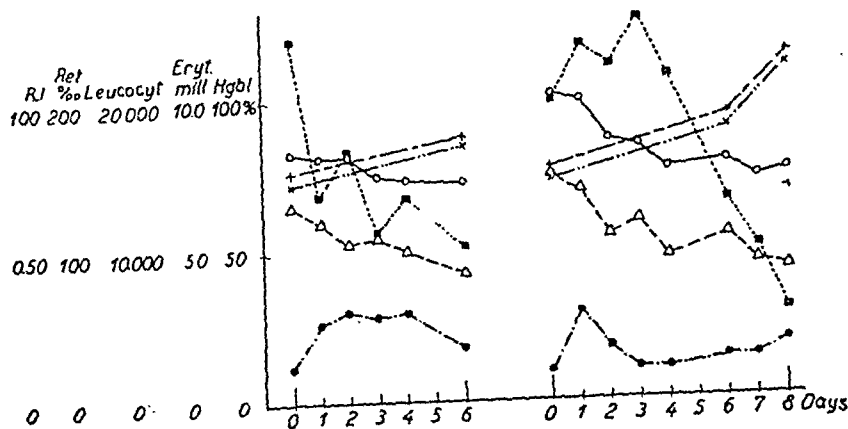


Fig. 3. Variations in the blood picture and ripening index during benzol poisoning.

- Percentage of hemoglobin (Zeiss Ikon).
- △—△ No. of erythrocytes in millions in cm^3 .
- No. of leucocytes in thousands per cm^3 .
- No. of reticulocytes in ‰.
- ×—× Ripening index for plasma.
- +—+ Ripening index for plasma plus tyrosine.

locytes increase in number during the first days after the injection, but then decrease. Even though the comparative rise agrees with e. g. Schilowa's findings, we have never in this laboratory seen reticulocytes in the amounts stated by that investigator. Examinations of normal animals show values ten times as low as those of Schilowa.

During the brief treatment with benzol an increase is seen (Fig. 3) in the reticulocyte-ripening substances of the plasma.

Section of the animals after 6—8 days' treatment with benzol distinctly showed the individual variations in the benzol tolerance. In some animals a hyperplastic bone marrow was found or a hyperemic bone marrow with numerous megakaryocytes, other animals showed a hypoplastic bone marrow with many dilated vessels. Everywhere numerous cells occurred with pyknotic nuclei in which the chromatin was gathered in rings or clumps.

All the animals, both those with a hyperplastic and those with a hypoplastic bone marrow, showed an increase in the ripening substances of the plasma.

In animals in which the injections were discontinued (Fig. 4) a distinct fall in the function of the bone marrow set in, as estimated by the peripheral blood picture; then after about 8 days' unaltered

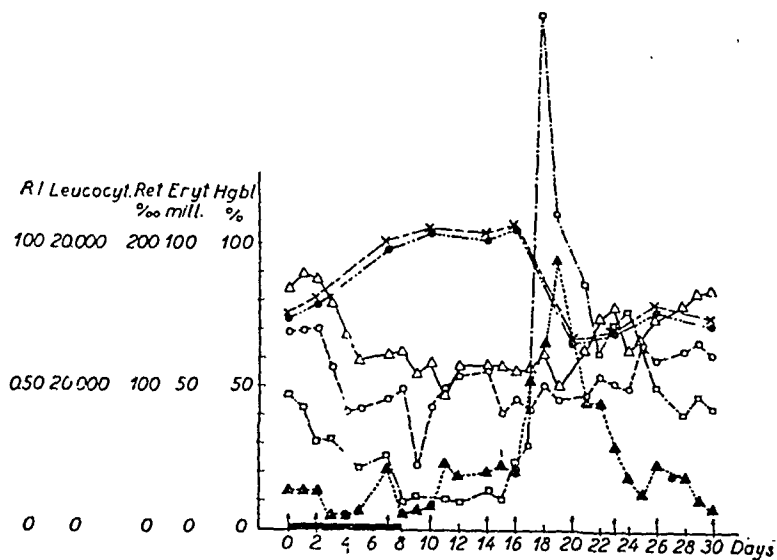


Fig. 4. Variations in the blood picture and ripening index during benzol poisoning and subsequent remission.

- △——△ Percentage of hemoglobin (Zeiss Ikon).
- No. of erythrocytes in millions in cm³.
- ◻—·—◻ No. of leucocytes in thousands per cm³.
- ▲——▲ No. of reticulocytes in ‰.
- Ripening index for plasma.
- ×——× Ripening index for plasma plus tyrosine.
- Injection of benzol.
- ↑↑ Taking of 5 cm³ blood samples.

blood picture a sudden rise occurred in the amount of leucocytes as well as reticulocytes, followed by a rise in the hemoglobin percentage and erythrocyte count. This sudden change in the blood picture must be supposed to be due to a sudden cessation in the blocking of the marrow.

The ripening substances which had greatly increased in amount as a result of the benzol poisoning kept this level until the above-mentioned blocking of the bone marrow had ceased, after which the amount of ripening substances fell to values below the normal, only to rise again to normal values, while at the same time the peripheral blood picture also became normal on other points.

Conclusion.

During benzol poisoning a reduction takes place in the function of the bone marrow, accompanied by an increased production of reticulocyte-ripening substances; conditions which are other-

wise observed when heavy demands are suddenly made on the ability of the organism to produce blood cells beyond the daily requirement.

Discussion.

On reviewing the various experimental anemias here described it will be seen that all the anemias investigated, no matter what their origin, are characterised by the occurrence of increased amounts of reticulocyte-ripening substances in the plasma. It has been pointed out several times that we interpret this as a link in the compensatory measures at the disposal of the organism for combating anemia. This agrees entirely with the fact that the amount of ripening substance is increased during the increase in the erythropoiesis in the early years of life [C. M. Plum 1943 (4) and Jacobsen & Plum, 1943].

Jacobsen & Plum have shown [1942 (1—2)] that tyrosine forms a part of the ripening substance complex. The addition of an extra amount of tyrosine to liver extract increases the effect of the latter on reticulocyte ripening, and C. M. Plum [1943 (6)] has shown that a tyrosine effect can be demonstrated not only on liver extract but also on extract or expressed juice derived from other organs. In the stomach especially there are substances which in themselves are inactive or but little active as ripening substances, but by the addition of tyrosine may be activated and affect the ripening of the reticulocytes.

It is uncertain which components within the ripening substance group are increased under the circumstances here described.

By blocking the reticulo-endothelial system in rabbits Jacobsen & Plum [1943 (3)] showed that the amount of ripening substances falls as a result, but that the plasma contains substances which can be activated by tyrosine.

It must be assumed, therefore, that substances are produced in the organism which are inactive in themselves but when coupled with tyrosine form substances capable of affecting the ripening of the reticulocytes.

In the forms of anemia investigated in this work it is seen that the increase in ripening substances is due to an increase of the whole complex as the addition of an extra amount of tyrosine is unable to cause a further activation of the ripening substances of the plasma.

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Renal Rickets.

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(Submitted for publication December 8, 1943).

Renal rickets (renal dwarfism, renal infantilism) is a disease, that was first described by R. C. Lucas in the *Lancet* in 1883. Since then cases with similar symptoms have been recorded particularly in America. In Sweden cases have been described by Faxén (26), T. Gordh & K. Kaijser (10, 4). In 1941 Rolf Kaijser gave an account of a case in the *Proceedings of Upsala Medical Association*. As it seems to be a rare disease, since there are many questions concerning it that are still unanswered both as regards the pathogeny and the clinical course, I should like to discuss it in connection with symptoms preponderant from the skeleton system and the kidneys. The changes in the skeleton are usually of the type that appear in rickets. The changes in the kidneys are often of a chronic nature and become visible both in early childhood and at any time up to puberty. It will now be regarded as fairly certain that there is a relation between the kidney injury and the skeleton process, although some investigators still doubt it. Several different forms of the disease have been described, but all of them have this combination in common viz. kidney trouble + disturbance in the development of the skeleton.

There is much to support the statement that a kidney injury is of primary significance in this peculiar disease. Thus it has been

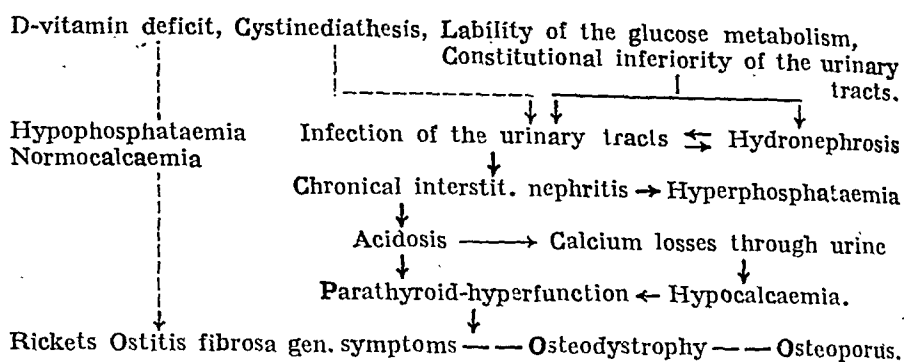
noticed that in most cases the kidney trouble appear long before the impairment of growth or the disturbance in the development of the skeleton. It is a known fact that it is possible to obtain a retention of phosphates in the case of kidney injuries. Thus hyperphosphataemia in renal rickets is easily understandable on a count of the kidney insufficiency. The kidney threshold for phosphates rises, and this also applies to other inorganic salts though not to such a pronounced degree. An acidosis must be consequence of this retention unless a compensation mechanism intervenes. The organism is normally equipped with several such, and these discreetly intervene guaranteeing an almost constant P_h . The so called ammonia defence of the kidney is considered to be one of these important mechanisms, by means of which the kidney thanks to NH_3 is able to compensate accumulated acids. This mechanism is rather sensitive, however, and cannot be maintained by an injured kidney and the result must be an uncompensated acidosis. There is however, another mechanism for compensation, but is not of the same effectivity as the so called ammonia defence of the kidney. The mechanism referred to is the liberation of the calcium ions of the skeleton, which can be brought about by means of the parathyroid hormone and this is just what seems to take place in the case of renal rickets. On account of the considerable claims thus made on parathyroid glands they hypertrophy. It is clear from extensive pathological-anatomical and clinical studies that parathyroid hypertrophy is a secondary claim. Several writers have been able clinically to point out an increased number of parathyroid hormones in the blood by means of Hamilton's and Schwartz's function tests. Since, as Albright and Ellsworth have shown, the parathyroid hormone also mobilises sodium-chlorine, increased values of these will also be obtained by means of hypertrophy. Full compensation is not achieved as a rule, which explains the reduced calcium values. Another explanation for the low calcium values is that as the phosphorus excretion via the kidneys become more difficult with the increased kidney insufficiency, this excretion must go instead via the intestine. This phosphorus there forms insoluble calcium phosphate compounds thus making the resorption of calcium more difficult or checking altogether (Mitchell). The final result of this is that the skeleton is exposed to a constant decalcification. Calcium deposits are also obtained inter-

stitially in the kidneys as a result of the hyperparathyreoidism which secondarily brings about parenchyma changes with kidney insufficiency. Thus a *circulus vitiosus*. It may be objected that in the case of hyperparathyreoidism no rickets develop without changes of bone formations as in *Ostitis fibrosa generalisata*. The following arguments may be raised however: Rickets do not always appear but sometimes osteodystrophy, osteoporosis and osteofibrosis.

The disease occurs during the growing period. As opposed to *ostitis fibrosa generalisata* rickets in its pathogeny is quite independent of the growing factor. As regards the speculations about an endocrine disturbance being the background to the dwarfism sometimes observed and the impairment of growth, it may be remarked that no genuine foundation has been able to be proved for a similar endocrine disturbance. The only endocrine disturbances that have been definitely established are those in the pancreas and the parathyroideae. Both dwarfism and the less serious impairment in growth may be explained by the rickets and the chronic acidosis that these patients often have. The sooner the disease appears the greater risks for serious impairment in growth.

The cystine diatheses have proved that constitutionally pre-supposed disturbances in the metabolism can produce symptoms resembling renal rickets. These cases warrant greater attention than has so far been given them. In these cases the kidney injury undoubtedly appears as a secondary symptom to the disturbance in the metabolism. As in other forms of renal rickets the kidney injury becomes an interstitial nephritis, which gradually develops into totally atrophied kidneys. All the symptoms occur that are typical for renal rickets. The impairment of growth and the dwarfism can be explained as a direct consequence of the cystine poisoning, as it has been possible to produce similar state in animals by means of experiments. The cystine diathesis also deserve attention, because the clinical diagnosis is so easily overlooked. *It ought to be made a condition that all cases of renal rickets should be tested to see whether cystinuria has developed and also that accessible organs such as the cornea and the conjunctiva should be examined to ascertain whether any crystalline deposits have formed.*

The writer looks upon the disease as developing according to the following lines:



Looking at this table it is clear that several cases that might be regarded as peculiar can easily be explained. Thus several writers have found normal or subnormal phosphorus values combined with normal to slightly increased calcium values. These cases should be regarded as early stages. The kidney insufficiency has not yet caused any noteworthy retention of phosphorus and therefore the ricket factor (the D vitamin deficit) possibly supported by an incipient hyperparathyroidism, naturally gives a normocalcaemia. It has been said that renal rickets also are different from the ordinary rickets in so far as the bone changes in the former case are resistant to vitamin D therapy. It will be found however, on examining literature that cases have been treated somewhat successfully with vitamin D. The effect has only been temporary, however, and in the majority of cases no improvement has been established. On the other hand this is no proof that there is no D vitamin deficit. The chronic kidney disease and the hyperfunctioning parathyroid glands which are constantly accumulating fresh calcium from the skeleton, are factors that subordinate rickets and make the cases incommensurable with the single D vitamin deficit. A lability in the glucose metabolism has been noticed in several cases. *It is desirable that the glucose tolerance test should be carried out in all cases of renal rickets since there may be no glucosuria and the blood sugar values may be all but normal.*

Own Observations.

The writer has had the opportunity of observing a case of renal rickets at the Medical Clinic of the Royal Serafimer Hospital. The patient visited the hospital on July 5th, 1943 for his knees, which were extremely deformed and it was almost impossible for him to walk. The father and mother were healthy and there was no relationship between them. Both were normally developed as well physically as mentally. About 10 years ago the father had had a kidney disease with albumen. A maternal uncle died of kidney disease with «uremia» about 3—4 years ago. The patient had no brothers or sisters. Apart from the kidney diseases there was no heredity of any interest.

The patient, a boy of 14, had grown up in Åland, where the father was a farmer. No complications had arisen at the confinement, and his weight then was 3200 g. After having mother's milk from the breast for a month the patient was weaned, owing to dyspeptic trouble and shortage of milk. The dyspepsia was very persistent during the whole of his childhood he was periodically troubled with indisposition and severe vomiting. When he was about 2 years age a doctor diagnosed albuminuria. He was put on diet, which was not strictly kept, however, for any length of time. In 1935 when he was 6 years old he had measles and in 1937 whooping cough. Both infections were of fairly short duration. In December 1938 mucous, purulent masses made their appearance in the urine, which also began to smell bad. Urine normal at times however. No temperature and no strain. Polyuria and polydipsia.

In 1939 he visited a children's hospital in Stockholm, and the following status was established: Height 130 cm (normal) weight 28.8 kg (— 1.2 kg). The general condition was unaffected, scratches on back. Flesh and musculature ordinary. Superficial lymph glands could not be palpated. Heart: nothing remarkable. Blood pressure showed normal value (120: 80 mm Hg) Lungs: nothing remarkable. Abdomen soft, not tender, no palpable resistences. External genitals normally developed. Cavity of the mouth: teeth 11/14 Pharynx: tonsils ordinary. In the urine about 1 ‰ albumen. The sediment, which was for the most part mucous greenish yellow to yellowish white, showed a fair abundance of white blood corpuscles as well as long gram negative bacilli. The phosphorous and calcium determinations in the blood showed 7 and 9 mg % resp. S. R. varied between 11—57 mm/hr. Hb remained at about 70 %. The number of red blood corpuscles was about 4 million. The water test showed isostenuria with a concentration capacity of only 1,012. Intravenous urographia did not fill the pelvis of the kidney. It thus seemed feasible to diagnose the existence of a kidney malformation almost completely without ureters and kidneys right on the bladder with an abnormal pelvis running straight into the bladder. Diagnosis established: Chronic nephritis as well as malformation of the urinary tracts.

Blood.

[illegible]

Date	Npn	Co ₂	P.	Ca.	K.	Na.	NaCl	Cl	A	cholest	sugar	phos- phatas	Ur ⁺	Ur ⁻ creat	Xant	Ind.	I ser.	Hb	red	w	SR
25											116										
28																87.	st. pos.				142
30	109	61	9.0	9.8					6.3												
31											138									5200	
3.9														9							135
6	184	46	8.3	10.6											8.8						120
10	139	51	10.8	9.4									102			82	st. pos.				
17	176	44	9.1	9.7															39	2.0	7000
21																					

N-pn = non-protein nitrogen CO₂ = alkaline reserve P = phosphates K = potassium Na = sodium NaCl = salt
 Cl = chlorine A = albumen cholest = cholesterol Ur⁺ urica Ur⁻ = uric acid creat = creatinine Xant = Xantine I = Indican
 I = iron of the serum, Hb = haemoglobin red = red bloodcorpuscles w = white bloodcorpuscles SR = sedimentation reaction.

When discharged from the hospital the patient was ordered to avoid salt and strongly flavoured food, and at the same time was told that he was suffering from congenital kidney malformation, which was incurable.

In 1941 he began to have trouble with his knees, which were so deformed that the under leg turned outwards in a more and more pronounced valgus position. He now began to have pains in his hips. In 1942 he sought medical help in Helsingfors, where they considered his case incurable. He went to hospital there, and after a few days he was discharged and ordered to take codliver oil and calcium. The state of his legs gradually deteriorated and it was almost impossible for him to walk.

Status after examination here on July 5, 1943.

Height 142 cm (14 years of age). Weight 32 kg. Musculature poorly developed. Skin pale with a shade of a sallow colour. Excoriateous all over the body partly fresh and bleeding. Eczematous changes on gluteal parts and over sacrum. On lower eyelids indications of oedema, Sclerae pale, not icteric. In repose fibrillary spasms observed in isolated groups of muscles in the upper extremities. Chvostek's sign positive. Foetor ex ore. In repose no dyspnea or cyanosis. Breathing frequency 16—20/min. Cranium of ordinary shape with slight lumps on the tubera. The costochondral junctions on ribs showed osteophytical lumps, nothing similar was noticeable in the epiphyseal of the long bones. Indications of Harrison's groove. Spine slightly right-convex. Scoliotic and kyphotic chest.

Genitals: Testes small, thin male hair growth, otherwise hair growth normal.

Cavity of mouth and pharynx: Large gaps between the teeth and enamel-defects in places.

Tonsils normal size, pale.

Thyroid gland of normal size and consistency.

Superficial lymph glands: some glands about the size of a granule in the corners of the cheeks.

For the rest no palpable lymph glands.

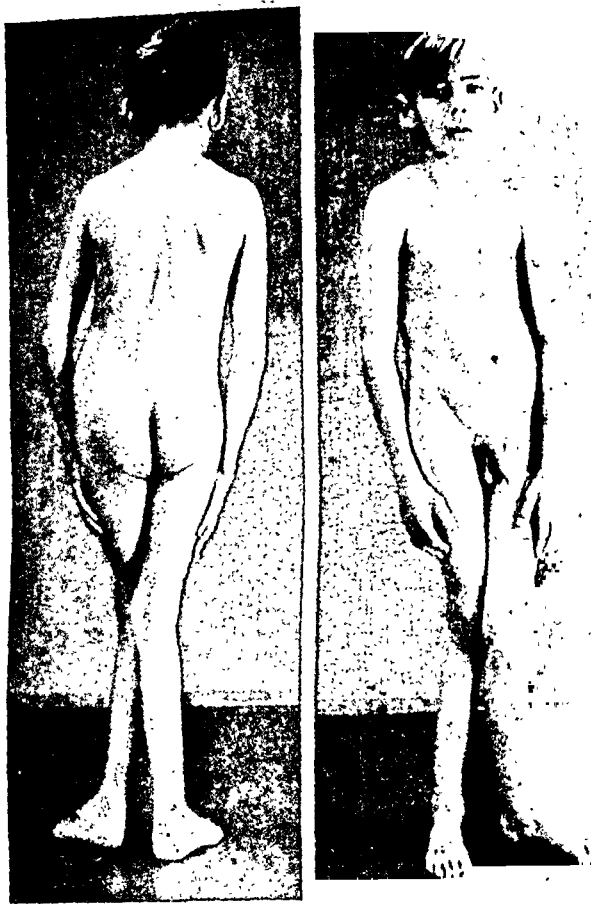
Heart: no signs of enlargement. Rhythm regular, slight systolic vesicular murmur with p. m. above the apex. No accent. Periphery vessels: nothing remarkable. Bloodpressure: 105/50. Pulse 100 beats pr. min. even and well filled, fairly gluth.

Electro-cardiogram: Nothing pathological.

Lungs showed physically normal finds, X-ray:

normal conditions. No calcium deposits. Abdomen: soft, not tender. Liver and spleen were not palpable. For the rest: no palpable resistences. Rectum: when palpating normal finds.

Reflexes: pupils wide, of equal size, reacted well to light and objects near at hand. Patellae, achilles, brachioradialis, cremaster and abdomen reflexes fairly active, Babinski negative bilaterally. Hep movements: Good mobility in all directions without any pain. Knee joints: pronounced gen. valg. bilat. with a good 30 cm between the medial malleolars, the insides of the knees touching each other. Free mobility in both knee-



Height 142 cm. Weight 32 kg.

joints, No tenderness, swelling or hydrops. Wasserman's reaction negative. S. R. varied between 94 and 142 mm/hr. during stay at hospital.

The faeces: firm sausage-like, dark brown, alkaline, 0 mucous, 0 undigested food remains, catalase negative, benzidine reaction negative no muscular fibres, iodophilic substance scarce, 0 iodophilic bacteria, neutral-fat scarce, 0 sebacid fat,

Urine: see continuation.

Examination of the eyes: Diagnosis hyperopia $S_v^h = 1.0 (+ 0.25)$.

The eyegrounds. nothing special, no crystalline deposits in the cornea or the conjunctiva.

Hearing test: heard whispering with right and left ear at 8 m. without any difficulty.

Cystoscope examination: The bladder was found to be covered everywhere with thick mucous scabs, which made it extremely difficult to get the organ clean enough to obtain a satisfactory view. After washing several times it was, however, possible to see a couple of normal ureter openings in normal places and the catheters were easily injected almost $\frac{1}{2}$

Urine.

Date	NH ₃	gm/dgm.			A.	G.	L.	H.	Ésb. ‰	P _h	Quan- tity ml.	Sp. g.	NaCl ‰	Sed.	Cys- time
		NH ₃	Ca.	Ur ⁺	Ur ⁻										
10.7								+	1.0	8.5	1400	1.009		solitary r. richly w	0
11								+	0.6	8.5	1000	1.005		" " " "	
13							—	+	1.0	8.5	900	1.011		" " " "	0
14								+	1.2	8.5	400	1.006		4-5 r. " "	
15			0.02					+	1.0	8.5	1000	1.006		solitary r. " "	
16			0.04					+		8.6	1800	1.007		" " " "	
20			0.24					+		alk.	1500	1.009		2-7 " "	
23			0.07					+	0.8	"	900	1.008		solitary " "	
26			0.07					+	0.8	"	2000	1.006		" " " "	
29			0.05					+	1.0	"	1800	1.006		Richly " "	
31								+	1.0	"	1500	1.005	2.1	solitary " "	
2.8								/+	1.0	"	1500	1.008		" " " "	
4								+	1.0	"	1400	1.007		" " " "	
6								+	1.0	"	1100	1.005		" " " "	
9								+	1.0	"	1400	1.006		" " plenty "	

meter into the bladder, Urine was obtained normally drop by drop from the left but nothing came from the right. Nothing pathological was observed in the mucous membrane of the bladder apart from the thick mucous layer; it was a normal colour and the bloodvessel were no fuller than usual. On X-ray in connection with the cystoscope examination showed contrasting catheters on the right side reaching up to processus transversus on L 3. A small quantity of contrasting liquid was injected in a distended pelvis, which was situated in an approximately normal position. As the patient was so weak, retrograde pyelography was carried out on the right side only. Intravenous pyelography was considered contraindicated owing to the uremic condition of the patient (Dr. Frykholm).

The skeleton X-ray on July 7th 1943 gave the following result:

coarsemeshed structure inside the bone of the cranium. Strikingly little appearance of all sutures. Normal Sella turcica.

Right hand: fairly coarse-meshed structure on the skeleton, which made the edges jagged inside some of the phalanxes. The epiphysic lines in the metacarpal bones were considerably broader than is usually the case of this age. The epiphysic lines in the distal ulnar part of the ulna were also broad. Chest and lumbar column: coarse-meshed and sclerotic vertebrae, otherwise no changes.

The pelvis and hipjoints: here too the bones are coarse-meshed and sclerotic. The sacroiliac joints are strikingly broad. The epiphysline in the proximal parts of the femur are considerably broader on both sides than is usually the case and are distinctly irregular. Both the femur shafts are higher up in relation to the caput epiphysis as in the case of epiphyseolysis. On the right side the displacement is 1 1/2 cm, on the left almost 1 cm.

Knee joints: Obvious valgus position in both knee joints. Here too the bone is coarse-meshed and sclerotic. The epiphysis lines in the tibia and the femur are distinctly broader than is usual in the lateral parts. (The concavity is to be found where the weight of the valgus position is greatest).

In the medial parts, on the other hand, these lines have a normal appearance.

Foot joints: here the bone is also coarse-meshed and sclerotic. The epiphysis lines are broad and irregular. On the left side the lateral distal part of the diaphyse of the fibula close to the epiphysis line is displaced somewhat laterally as after an epiphyseolysis with fracture. No obvious misplacement. No distinct corticalis appears in the parts of the skeleton photographed owing to the bone being coarse-meshed.

The changes coincide with those seen in renal rickets and correspond in the main with Parson's Stage 3 (Holmgren).

As it was of interest to ascertain whether a hydronephrosis also existed on the left side, a fresh cystoscope examination with retrograde-pyelography was carried out on this side, and a distended and clumsy pelvis was seen. Thus double hydronephrosis had been proved by X-rays.

The kidney function.

As a rule the quantity of urín pr 24 hrs amounted to about 1500 ml. The specific weight showed an isostenuria with values fixed at round about 1,005—1,009. The reaction was alkaline, except for a time when bicarbonate was not given. A quantity of $1/2$ —10 % of albumen was constantly found in the urine. The sediment showed from moderate to considerable quantities of white blood corpuscles, occasionally red ones and also an abundant flora of staff-shaped bacteriae. During the time of observation the blood pressure varied from 105 to 135 systolic and 50—70 mm Hg diastolic. As will be seen from the tables and diagrams there was a remarkable retention of nitrogen substances. Creatinine clearance showed a striking decrease below the normal values, and on one occasion it was only 14 cm³/min.

There was a substantial increase of non-protein nitrogen substances in the blood, which will be seen from the diagram table. In addition to this abnormally increased values could be shown of xanthine protein as well as the existence of indican. No noteworthy variation of the xanthine protein retention occurred during the time of observation which took over 3 months. Instead it is striking that no congruity with the non-protein nitrogen evidently exist.

The non-protein nitrogen values varied between 110 and 200 mg % during the time of observation. Even while at the children's hospital in 1939 the patient had about 90 mg % non-protein nitrogen, therefore it may be rightly said that he had had abnormally high non-protein nitrogen values for at least 4 years. This non-protein nitrogen retention has in all probability been counterbalanced by a more or less pronounced state of acidaemia. Fig. 1 undoubtedly shows an inverse proportionality between the non-protein nitrogen values and the alkaline reserve (measured in vol % CO₂ in plasma). On arrival the quantity of CO₂ was as low as between 20—30 vol %, which proved the existence of marked acidaemia. The non-protein nitrogen values were high: Ca 190—200 mg %. Sodium bicarbonate was then given in a large dose of 15 mg pr. day, whereupon the quantity of CO₂ immediately rose to the normal 60—70 vol. % (Van Slyke). The non-protein nitrogen values gradually sank but more slowly. The patient was given

plenty of liquid every day ca 2000 ml from the time of arrival. A simultaneous determination of the blood albumen showed that there was no hydraemia to explain the decreasing nonprotein nitrogen values. By giving and stopping the sodiumbicarbonate it was possible to determine the quantities necessary for the balance. It turned out to be about 9 g pr. day. By keeping this quantity of sodium bicarbonate + 3 g Calcido the best balance was obtained with a normal alkaline reserve and the lowest non-protein nitrogen values that could be noticed during the whole time that the patient was under observation (non-protein nitrogen 110 alkaline reserve 60). When stopping the sodium bicarbonate on Sept. 2 nd. the non-protein nitrogen values immediately rose from 110 to 180, while the alkaline reserve sank from 60 vol. % to 45 vol. %. Thus the use of sodium carbonate then causes an immediate rise in the alkaline reserve and a fall in the non-protein nitrogen. The effect is quite obvious and the state of equilibrium seems to be extremely sensitive to the external supply of alkalis. The almost immediate effect is striking. As has already been mentioned, the mineral metabolism is particular meet's with disturbances owing to the kidney insufficiency. The writer has discussed the relation between phosphorous and calcium values and has pointed out that calcium values and increased phosphorous values are by no means compulsory for the disease, as it depends witerely upon how largely the compensation mechanism can influence the fixation of the equilibrium, If the kidney insufficiency is less pronounced, while the ricket element is more so, the result may simply be inverse, i. e. decreased phosphorous and inceased calcium values. The writer's case shows clearly increased phosphorous values during the whole time the patient was under observation, whereas the calcium values are normal. The normal calcium values seem to be due to the fact that sufficient compensation mechanism (parathyroidae) work in favour of normal equilibrium and that there is a sufficient supply of calcium (medication, the skeleton). The obvious effect of the administration of calcium both intravenously and pr. os. (glucosate of calcium and calcido), are worthy of notic. Calcium values which in other cases usually show extremely slight variations, now present a distinct increase with the use of calcium (see fig. 1). When calcium was again given on Sept. 24 th the calcium values of the blood fell immediately. Looking at fig. 1 there are still more

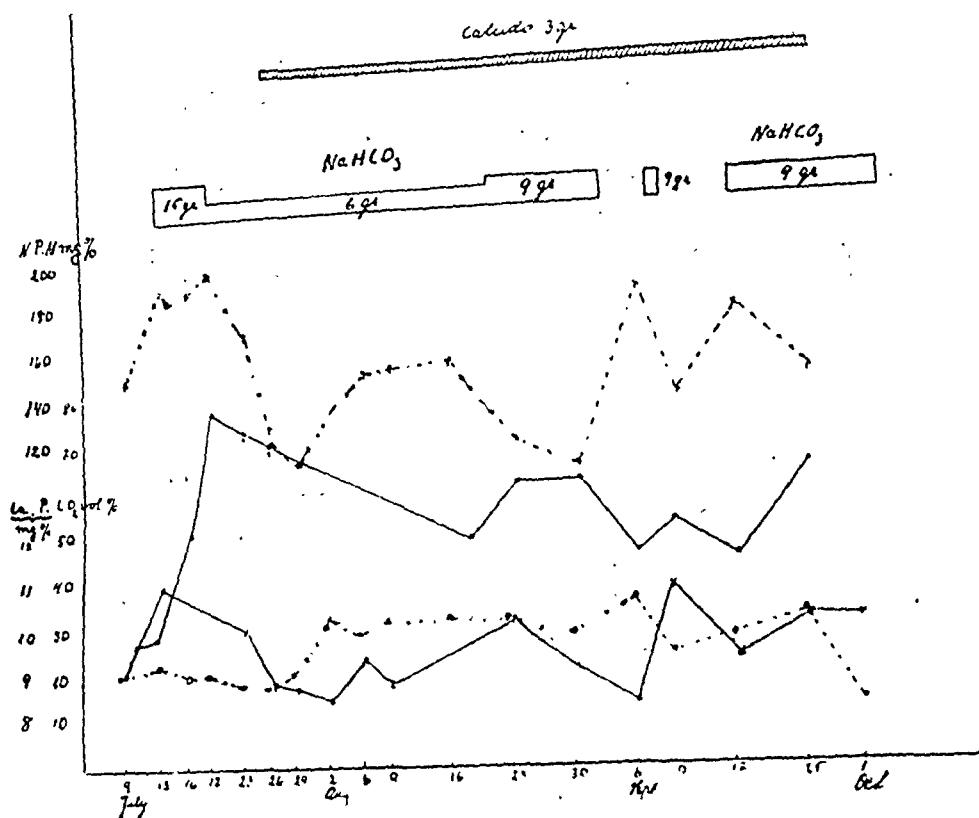


Fig. 1.

- x — — — — x Non protein nitrogen mg%.
- o — — — — o Alkaline reserve, vol.% CO₂ in plasma.
- x — — — — x Calcium mg.%.
- o — — — — o Phosphates mg.%.

facts of interest, there is an obvious congruity between the non-protein nitrogen and the phosphorous values, although the variations in the non-protein nitrogen curve occur some days later than those in that of the phosphorous. As acidaemia causes increased disintegration of the tissues and thereby an increase in the non-protein nitrogen but at the same time also involves a mobilization of the organic salts in the skeleton, it is possible that there exists a genuine relation between the two.

Fig. 1 also shows that there is a certain proportionality between the calcium and phosphorous values, which was also to be expected.

»The ammonia defence«

Since the so called ammonia defence of the kidney is one of the important compensation mechanisms when acidæmia develops, it was interesting to see how this mechanism functioned. It proved (fig. 2) that giving sodium bicarbonate caused no increase in the ammonia excretion in the urine despite the fact that P_H sank to 6.7. The excretion of ammonia was very low the whole time, which may be partly due to the strict kidney diet that the patient had kept. That the patient's ammonia defence played no important part as compensation mechanism, however, is perfectly obvious in fig. 2 from which it will be seen that the average ammonia excretion was not more than about 0.1 g pr. 24 hrs. The normal value with a mixed diet is stated to be 0.7 g pr. 24 hrs with the marginal values 0.3—1.2 g.

It is, however, clear that there is a certain ammonia defence, though it does not function when the patient is on ordinary kidney

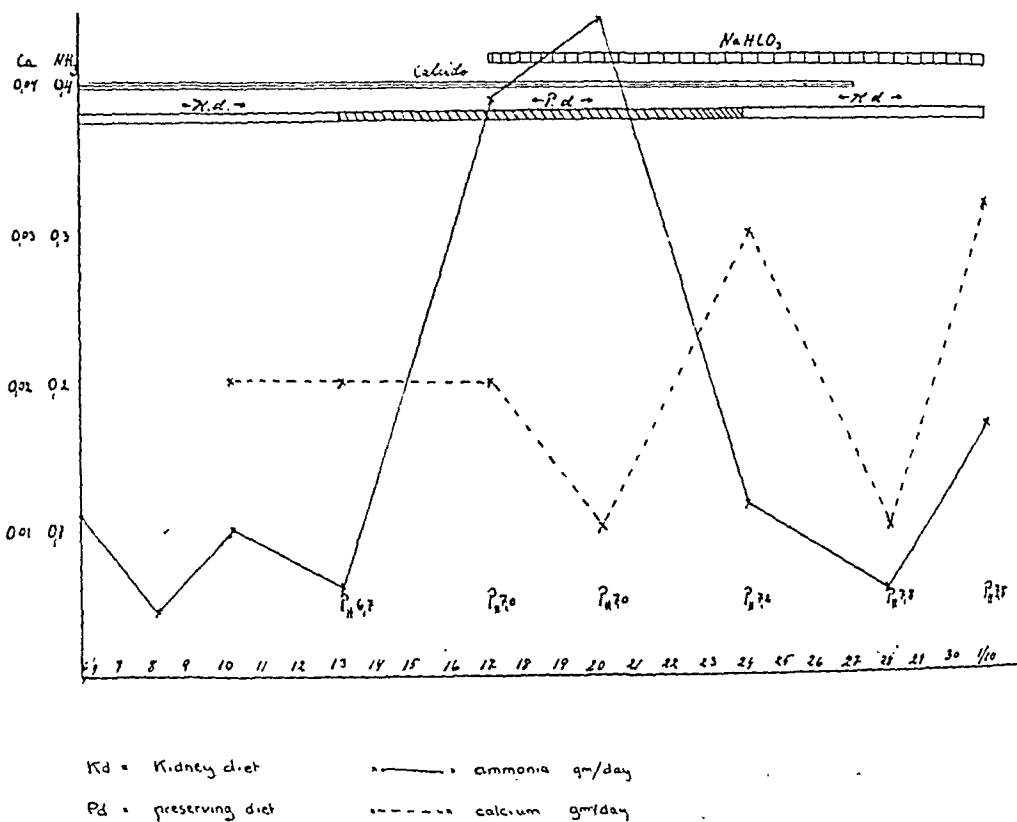


Fig. 2.

diet. When giving a less strict diet there will be an immediate increase in the ammonia excretion (see fig. 2). By giving sodium bicarbonate, however, the ammonia excretion drops to the lower level. At the same time P_H in the urine rises to 7.8. From this experiment the conclusion may be justifiable that sodium bicarbonate plays a considerable part in combating acidemia. The significance of diet for the insufficient kidney is also demonstrated. Thus the most suitable therapy is kidney diet and bicarbonate, naturally under the existing circumstances with a chronic uremic state in a young and growing person there is a temptation to endeavour to moderate the rigorous diet somewhat, but from the discoveries made, it seems scarcely advisable. While the patient was on a fairly free diet he was troubled with subjective discomforts in the form of headache and indisposition. The non-protein nitrogen and the alkaline reserve on the other hand, showed no noteworthy variations during the time he was on this diet.

The Nitrogen Equilibrium.

What is of particular interest in this case is the fact that for years the patient had been able to go with a nitrogen reserve up to 100 mg %. The question must undoubtedly arise how anyone could live for so long in such a state of infection. As has already been pointed out, however, of the intermediary refuse products of the albumen metabolism, urea, which forms the main part of the non-protein nitrogen, is of little significance as far as the origin of the urimic symptoms is concerned. The poisonings are more parallel with the appearance of cyclic disintegrating products from albumen or putrefactive products from the intestine (phenols, aromatic amino and oxy-acids, Becher). Thus even if urea cannot be considered the immediate retention product producing uremia, its accumulation in the blood is nevertheless of such pronounced magnitude that it must have injurious effect in the long run.

Accordingly it is known that poisons penetrate more easily into the cells when the percentage of urea in the blood is high. Moreover, in the case of increased kidney threshold urea is excreted everywhere with the digestion fluids owing to its solubility. In the stomach and the alimentary canal it becomes ammonia and this injures the mucous membrane. This causes uremic gastritis, ente-

ritis and stomatitis. It has even been supposed that urea might promote the retention of phenols.

The examination of the excretion of urea in the patient's urine showed so low a value as 3 g urea nitrogen pr. 24 hrs. which approximately corresponds to 6 g urea pr. 24 hrs. When the normal value for excreted urea in a grown-up person is round about 20—30 g pr 24 hrs, the question arises as to what became of the rest. It must be admitted that the patient is not grown up and weighs only a little over 30 kg. and he also keeps a strict kidney diet, but in spite of this the value seems far too low for an ever increasing retention of nitrogen substances not to gradually appear, unless there is some mechanism for the elimination of the nitrogen substances. The examination of the urine also showed that no other nitrogen substances were excreted to any great extent, therefore it seemed impossible for a compensation to take place in this manner. Accordingly there was only about 0.15 g uric acid pr. 24 hrs. Reckoning with ca 10 g urea pr. 24 hrs as being a normal excretion for anyone of the same age and weight as the patient and keeping the same diet, ca 4 g, however, was retained and accumulated in the body every 24 hrs, unless some mechanism capable of excretion and functioning for the kidney were at work. This would mean considerably more than 1000 g pr annum, which seems absolutely out of the question remembering that he had been suffering from a serious kidney insufficiency and uremic symptoms for at least 4 years. There must therefore be substituting organs for excretion. In theoretically the following are possible. 1) the alimentary canal 2) the lungs 3) the skin and mucous membranes. The writer has examined all these organs in order and has found the following noteworthy facts:

In the circulating blood there was urea in a concentration of over 100 mg, i. e. an increase of 5—6 times of the normal.

The faeces: These showed a quantity of urea varying on different occasions from 51—88 mg % corresponding to 102—176 mg % urea.

If the quantity of faeces for 24 hrs is reckoned as being 500 g, a maximum of 0.9 g pr 24 hrs would be disposed of in this manner. This is naturally a considerable quantity, when a normal calculation shows that there are only traces of urea visible on faeces. It is easy to imagine the mechanism for an increased quantity of urea in faeces. As urea is soluble, it follows into the digestion fluids

in cases of concentration, e. g. gall, intestinal juice etc. As has already been mentioned, some of the urea may be disintegrated in the alimentary canal, thus producing ammonia. According to an old observation there are said to be ureas in the ventricle, which may possibly disintegrate the urea so that part of it might disappear with the intestinal gases or be removed through the ructus (43). As regards moment 1 it has accordingly been shown that the alimentary canal place the part of substituting organ for the kidney in the nitrogen excretion. The quantity concerned (ca. 0.9 g pr. 24 hrs.) is, however, not sufficient. Consequently moment 2 was investigated.

The lungs: No case is described in literature in which urea or any other nitrogen substances have been proved to exist in the exhaling air. According to physiological thinking it is plausible that the lungs might function as a substituting organ, just as it is an old acknowledged fact that the breath of uremipatients smells «uremic». There will nevertheless be many cases when the uremic smell established has concerned a stomatitis or gastric uraemica which produces foetor ex. ore. The mechanism for an excretion via the lungs may be thought to function either:

1) through the excretion of passing nitrogen substances (NH_3 , amines) 2) the excretion of urea, which may possibly be dissolved in drops of water in the exhaling air.

3) the urea is disintegrated by bacteriae to NH_3 and thus removed. The first is the most likely of these three alternatives, as to no. 2, it is theoretically possible but not likely that any great quantities would be removed in this manner. No. 3 is hardly likely, as it would presuppose an invasion of bacteriae far down in the bronchials if it were to play any important part.

The following method was adopted for the investigation. A quantity of 95 ml distilled water and 5 ml 10 % H_2SO_4 so as to obtain a solution of 0.5 % was poured into a washing bottle with one of the glass tubes dipped down to about 1 cm above the bottom. A rubber tube and a small glass tube loosely filled with cotton wool was attached to the glasstube dipped down into the solution. The patient was now instructed only to breathe through this rubber tube, when the H_2SO_4 solution got full of bubbles thanks to the exhaling air. After ca 700 exhalations the nitrogen substance in the solution was determined.

It then proved that there was nitrogen in the air exhaled in a considerable quantity considering the patient. Reckoning 15 breathes per min. will give 21600 breath pr. 24 hrs. The total quantity of nitrogen turned out to be about 400 g pr. 24 hrs, or if calculated as urea 0.75 g pr. 24 hrs. Control tests with a corresponding member of breaths from a healthy person, a kidney patient (acute nephritis on kidney diet having no non-protein nitrogen increase) as well as tests of room air in a volume corresponding to 700 breaths showed no existence whatsoever of nitrogen substance. Accordingly a substituting excretion of nitrogen substances via the lungs was proved in the form of NH_3 , amounting to such large quantities as ca. $\frac{3}{4}$ mg pr. 24 hrs. (calculated as urea). There was no proof of methylamine.

The skin: As it is a well known fact that the skin exudes uric acid and urea as well as creatinine and ammonia, in some cases in rather considerable quantities, an investigation was also made of the nitrogen exudation from the skin. It was carried out in the follow manner. The skin was not washed for a certain number of days, after which the upper part of the body was washed with water, which was kept and sent to be analysed. The total quantity of nitrogen amounted to 0.297 g. Of this 0.019 g was NH_3 nitrogen. There was 0.150 g urea nitrogen and other nitrogen amounted to 0.129 g. If the total quantity is reckoned as urea then 0.594 g was obtained. As only about half the surface of the body was washed, the real quantity is dubbel or ca. 1,199 g. This quantity had been collected thanks to no washing for 5 days, thus ca. 0.23 g urea pr. 24 hrs. as an equally large quantity had in all probability steck to the clothes. The figure may be dubbed. *The result is that about $\frac{1}{2}$ g is exuded from the skin pr. 24 hrs.* The following table shows the quantities found.

Normally	urea	Patient I. K. urea
Urine	Ca. 10 gm.	ca. 6 gm.
Faeces	traces	» 1 »
Lungs	—	» 1 »
Skin.....	variable (usually only traces)	» 0.5 »
Total	ca. 10 gm.	ca. 8.5 gm.

As of course 10 g pr. 24 hrs. as a normal value is only approximate and largely dependent on the diet, it must be said that 8.5 g pr. 24 hrs. probably represents a normal value. The sources of error when making a quantitative estimation of the nitrogen excretion must also be considerable, inter alia the exudation from the skin is problematic may be far greater than these figures show, so that 8.5 g pr. 24 hrs. may be regarded as representing a normal value. In any case the main object has been achieved i. e. a substituting nitrogen excretion of considerable magnitude has been shown, primarily from the lungs but also from the digestion canal and the skin. It has also been explained that a patient has been able to live and keep fairly well for years despite a defective excretion of nitrogen substances via the kidneys.

The function of the liver:

The determination of the magnitude of the red blood corpuscles was used for this, it being at present the most reliable proof of the function of the liver. (See coming article by G. Hammarsten). The average diameter showed a value of 7.6μ , which is of course a normal value.

Endocrine organs:

The pituitary: The cranium X-ray showed a normal sella turcica. No sign of calcium spots in the place for the pituitary. There were no finds in the status of the patient to indicate any pituitary disturbance.

Thyroidea: In the case that K. Kaijser published in 1940 there was low basel metabolism: values being — 17—21 %, otherwise the thyroid function is usually normal with the disease. In this case the values of + 19 % and + 18 % were obtained in the course of a month. (Values calculated according to prof. of Nat. Ac. of Sciences 6, 1920 p. 6.)

Parathyreidae: No roentgenological visible enlargement could be seen. But in all probability there were hyperplasia and hyperfunction of the glands, as is usually the case with this disease. In fatal cases a similar hyperplasia has most often been proved. No attempt was made to test the function according to Hamilton (11), as this method gives somewhat uncertain results.

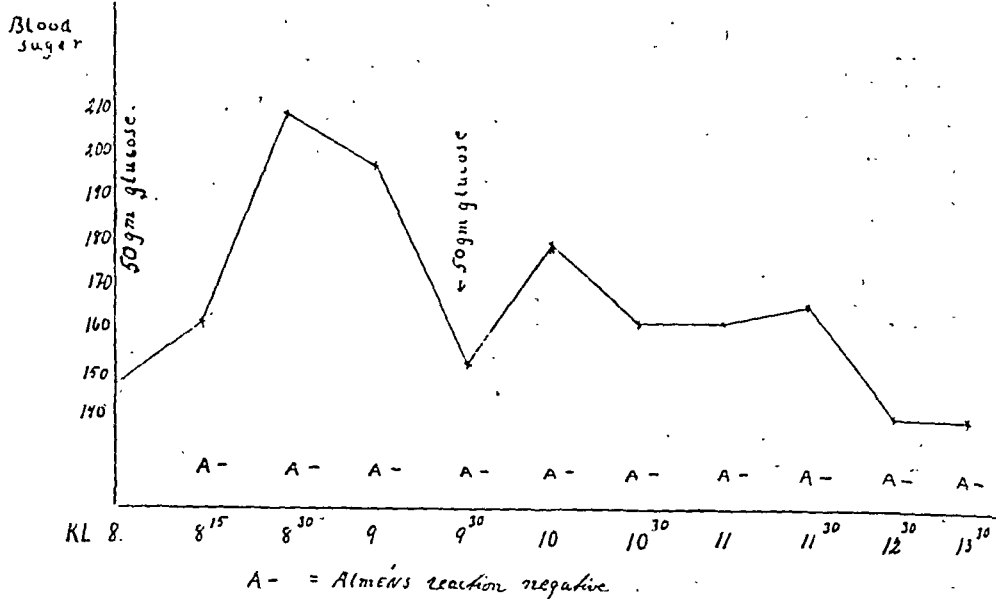


Fig. 3.

A- = Almén's reaction negative.

The endocrine function of the pancreas as well as superior organs: As has already been mentioned, renal rickets is sometimes combined with glucosuria. In several cases this has proved to be of a diabetic type. It cannot be denied that a lability in the glucose metabolism, which at times manifests itself in the form of glucosuria is fairly common with this disease. Bearing in mind that there is so often a pyonephrosis, the question arises whether an intermittent glucosuria is not largely the cause of the infection in the urinary tracts. In this case no glucosuria developed during the time of observation. When examining the patients blood values at different times during the 24 hrs, strikingly high values were found. This hyperglycaemia was found on repeated examinations of the blood sugar during the variations of the 24 hrs. When examination were carried out on three different occasions all the fixed values were above 150 mg %. According to Staub-Traugott (double tolerance tests) showed the type as is seen in fig. 3. It is thus uncertain whether there has been any insufficiency in the endocrine function of the pancreas.

Adrenals: It has been mentioned that the mobilisation of calcium from the skeleton has been thought to make calcium deposits form easily in certain parenchymatous organs such as the lungs, the kidney and the adrenals. The X-ray of the lungs showed no

calcium deposits. The surveys of the kidneys showed no calcium deposits either, nor was there anything in the place for the adrenals. The patient's skin was sallow and as has already been said, this is supposed to be due to an insufficiency of the adrenals owing to calcium deposits.

The mechanism is, however, probably different.

Sexglands with superior organs: Considering the patient's age, the secondary sex characters must be regarded as being fairly well developed. The puberty hair was, however, perhaps somewhat scarce. The testes and the epididymis were normal. On several occasions there was sperm in the urine which seemed to be normally developed, and from all this it seems evident that no obvious disturbances in the functions of the endocrine organs have been able to be proved.

The blood: This was of a secondary anaemic type. As was to be expected, there was no improvement whatsoever after treatment with iron. It is not surprising in this case as the anaemia is in all probability of a secondary nature as compared with the accumulation of phenols and poisonous desintegration products from the albumen. On one occasion there was a blood transfusion when 350 cm³ was given, but this had no apparent effect. The resistance test of the red bloodcorpuscles showed a maximum resistance of 0.34 % and a minimum of 0.51 %. The infection of the urine tracts: On two occasions an aerobically growing bacteria was obtained from both bladder and ureters. The morphology of which resembled mushrooms.

Prognosis and Therapy.

Where urimic symptoms are concerned, the prognosis must in every case be a pessimistic one. It is amazing, however, how these patients can live year after year in a sub-uremic or entirely uremic state. The writer has pointed out the compensation mechanisms and substituting organs that function and prolong life.

As is always the case the prognosis depends on at what stage the disease has been diagnosticated. When the skeleton symptoms appear comparatively early, it is naturally difficult to diagnose renal rickets. But when the kidney disease is the primary and principal were both prognosis and therapy coincide. A strict treatment of

the usual affections occurring in the urinary tracts begun in good time will be of decisive significance unless hydronephroses or malformations make their appearance. In the case of a single hydronephrosis or malformation, there are naturally indications in favour of an operation, provided of course that the patient can stand it. In this case with double hydronephrosis and secondary totally atrophied kidneys no urinary therapy was possible. What in all probability can prolong life is an immediate use of alkali in the case of acidaemia, as this treatment will lessen the disintegration of the elements of the tissues, the accumulation of injurious substances in the blood will be reduced, and death from uraemia will not be so imminent. In cases where there is a predisposition to oedema care must be taken in the use of alkaline. The writer has shown that the effect of alkali pr. os. is immediate as well on the non-protein nitrogen values. The patient also notices subjectively the improvement after the use of alkali. It is striking the fairly large quantities and repeated doses he can stand without having any trouble in the form of indisposition. The suitable dose pr 24 hrs. can be said to be about 9 g. It has also been seen that a continuous and strict kidney diet is essential in cases so far gone as the one mentioned. Anti-ricket treatment in the form of vitamin D with 15000 I. D. per day has been given for about one month, but no effect has been able to be established. Literature only mentions a couple of cases in which such therapy has met with any success. Of other vitamins, B₁ and C, have been given in order to facilitate resorption, primarily iron. This has been given in as large doses as 0.5—1 g \times 3 without there being any effect at all. Calcium has been given in the form of Calcido in a dose of 2 tablets, \times 3 and distinct effect could be noticed in the calcium of the blood. Thus calcium treatment is to be recommended, as by making use of it decalcification can probably be retarded somewhat and perhaps even checked.

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Summary.

1) The pathogeny and symptomatology of renal rickets is discussed. This disease should be looked upon as a syndrome, not as a unitary disease. The writer points out the similar disease arising when disturbance occur in the cystine metabolism. Con-

sequently all cases of renal rickets should be examined with reference to this disease.

2) The most important momenta of the origin of renal rickets are arranged in a schematic exposition. The most important factor in the renal ricket syndrome is the kidney insufficiency.

3) The writer discusses the origin of acidosis and hypocalcaemia.

4) The writer reports his own observations in a case of renal rickets.

5) The so called ammonia defence as well as the nitrogen excretion were studied in particular. There was proof of a considerable compensatory excretion of nitrogen substances through the lungs, the alimentary canal and the skin. The nitrogen substances excreted through the lungs were ammonia. There was no proof of methylamine.

6) The functions of the endocrine organs have been examined, but no signs of any noteworthy disturbances have been proved, apart from a certain lability in the glucose metabolism.

7) Prognosis and therapy have been discussed.

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Aus der Orthopädischen Klinik zu Lund (Schweden).

Die Hormonausscheidung bei chronischer Polyarthrit des Weibes.

Zugleich ein Beitrag zur Beziehung zwischen Geschlechtsfunktion und Gelenkleiden beim Weibe.

Von

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Dozent.

(Bei der Redaktion am 10. Dezember 1943 eingegangen).

In der Diskussion über die Ätiologie der sog. rheumatischen Polyarthrit hat in den letzten Jahrzehnten die *Fokalinjektionslehre* im Vordergrund gestanden. Wie man sich im übrigen auch zu dieser Lehre stellen mag, so muss man jedenfalls zugeben, dass klinische Erfahrung und experimentelle Forschung gezeigt haben, dass sie allein nicht in *allen* Fällen von chronischer Polyarthrit das Gelenkleiden hinreichend zu erklären vermag. Man hat in mehreren Gebieten der Medizin nach ätiologischen Momenten gesucht. Am fruchtbarsten erscheint mir der Gedanke einer Korrelation mit Störungen des *endokrinen Systems*. Hinsichtlich des Zustandekommens eines solchen Zusammenhanges sind die Ansichten stark geteilt. Manche Forscher wollen die Störungen des inkretorischen Systems als alleinige Ursache des Gelenkleidens verantwortlich machen, andere erblicken in ihnen nur beitragende und prädisponierende Faktoren, wieder andere wollen sie nur als Nebenerscheinungen oder sekundäre Folgen der Gelenkverände-

rungen gelten lassen. Grössere Einigkeit herrscht in bezug auf den Sitz der Störungen im endokrinen System. Zwar hat man den ätiologischen Schwerpunkt mal in dem einen, mal in dem anderen Bestandteil des Systems oder auch in pluriglandulären Kombinationen gesucht, in erster Linie aber hat sich die Aufmerksamkeit auf die hormonal gesteuerte weibliche Geschlechtsfunktion gerichtet. Man stützt sich dabei auf alltägliche Beobachtungen — das Überwiegen der Frauen in der klinischen Statistik, die Häufung von Fällen in gewissen Altersgruppen usw. So gut wie jeder, der mit einem grösseren Krankengut von chronischen Polyarthritiden zu tun gehabt hat, wird dann und wann einen auffallenden zeitlichen Zusammenhang zwischen dem Auftreten der Gelenkerkrankung und einer Menstruation, einer Schwangerschaft oder einer normalen oder künstlich hervorgerufenen Menopause festgestellt haben. Besonders der Zusammenhang mit der Menopause ist oft so eindeutig, dass man Begriffe wie »Arthropathia ovaripriva« (Menge 1924), »menopaus arthralgia« (Hall 1936), »Rheumatism ovarien« (Vignes 1937) u. a. geschaffen hat. Verschiedentlich hat man auch Fälle dieses Typs von den gewöhnlichen Polyarthritiden abgrenzen und sie eine ätiologisch freistehende Gruppe bilden lassen wollen. Im skandinavischen Schrifttum haben besonders Gram (1930), Rasmussen (1936), Kahlmeter (1937) und Snorrasson (1943) über einschlägige Fragen gehandelt.

Indessen liegen nur wenige experimentelle Untersuchungen zur Beleuchtung dieser Probleme vor, und exakte klinische Daten sind in der einschlägigen Literatur selten. Der zuerst beobachtete und bisher am besten untersuchte Zusammenhang zwischen Gelenken und Hormonen ist der erstaunliche Effekt, den Injektionen einer Reihe von zu den Sexualhormonen gehörenden Stoffen auf die Beckensymphyse von Meerschweinchen und Mäusen haben (Hisaw 1927, Fremery, Kober u. Tausch 1931, Tapfer u. Haselhofer 1935, Gardner 1936, Gardner u. Pfeiffer 1938, Sutro u. Pomerantz 1939 u. a.). Das Ehepaar Silberberg hat in einer Reihe von Arbeiten (1938, 1939, 1940, 1941) die Einwirkung verschiedener Hormone auf Gelenkknorpel und Knochengerüst von Meerschweinchen und Mäusen verschiedenen Alters untersucht. Ihre Experimente zeigen, dass Störungen des geschlechtshormonalen Gleichgewichts bei den Versuchstieren sowohl Arthritiden als Arthrosen hervorzurufen vermögen. Besonders wichtig scheint der Befund zu

sein, dass, während Follikulin bei jüngeren Tieren, in grossen Dosen verabfolgt, kaum regressive Veränderungen des Gelenkknorpels bewirkt, bei älteren Tieren starke derartige Veränderungen auftreten, was Silberbergs als Zeichen prämaturnen Alterns betrachten. Follikulin in grossen Mengen übt indessen auch eine Wirkung auf das Skelett aus; die Markhöhlen werden nämlich invadiert und grösserenteils mit Knochen ausgefüllt, Veränderungen im Sinne einer generalisierten Osteosklerose (Zondek 1937, Gardner u. Pfeiffer 1938, Sutro 1940). Diese Veränderungen vollziehen sich beim Männchen langsamer und lassen sich durch gleichzeitige grosse Gaben von Testosteron verhindern (Wentworth, Smith u. Gardner 1940). Hills u. Weinberg (1941) haben diese Erfahrung für die Klinik nutzbar gemacht, indem sie bei Fällen mit schlechter Frakturheilung Follikulin verabfolgten und so verstärkte Kallusbildung und Heilung erzielten. Jonsson (1939) hat die Frage des Zusammenhanges zwischen Geschlechtsdrüsen und Gelenkleiden experimentell zu beleuchten versucht. Er kastrierte Kaninchen — die Kastration an sich liess die Gelenke unbehelligt — und rief bei diesen mit Injektion von Serum, Tuberkulin oder Bakterienstämmen verschiedener Art Gelenkveränderungen hervor. Die Ergebnisse sprechen nach Jonsson dafür, dass endokrine Faktoren vom Schlage der Kastration als prädisponierendes Moment beim Zustandekommen der Gelenkleiden eine Rolle spielen können. Die experimentellen Untersuchungsbefunde scheinen sich dahin zusammenfassen zu lassen, dass sie eindeutig für einen Zusammenhang zwischen dem Knochengelenksystem und den Geschlechtshormonen des Weibes sprechen.

Klinische Beobachtungen betreffend die Korrelation zwischen Geschlechtsfunktion und Gelenkleiden sind wie gesagt alltäglich, doch finden sie in der Literatur nur spärliche Behandlung (De Sa 1937, Hall 1938, Hench u. Mitarb. 1939/40 u. a.). Noch seltener sind exakte klinische Daten. Soweit es sich unter den augenblicklichen Verhältnissen feststellen liess, ist Rasmussen (1936) der einzige, der durch exakte Bestimmungen des Follikulintiters im Harn von Polyarthritispatientinnen einen Massstab der endokrinen Störung zu finden versucht hat. Er sah bei seinen Patientinnen eine hochgradig verminderte Follikulinausscheidung sowie am Anfang der Behandlungsperiode Follikulinverhaltung. Rasmussen legt seine Versuchsergebnisse dahin aus, dass Follikulin

ein für die normale Gewebsentwicklung notwendiger Stoff und die polyarthritische Gelenkveränderung ein Ausdruck der durch Follikulinmangel verursachten krankhaften Zersetzung ist. Er betrachtet also die mangelhafte Follikulinproduktion als den primären ätiologischen Faktor. Eine interessante Ergänzung dieses Resultats ist die letzthin gemachte Beobachtung, dass das Blut von Schwangeren bei Polyarthrititis eine merkliche Heilwirkung besitzt (Basch 1941, 1943, Sjöberg 1943). Damit werden dem Organismus ja grosse Mengen Follikulin zugeführt, doch ist es noch ungeklärt, ob die Wirkung ausschliesslich darauf beruht, dass möglicherweise von der Hypophyse her auch ein »chondrotroper Faktor« mitspielt, oder ob andere, nichthormonale Faktoren sich geltend machen. Sundelin (1936) hat einen anderen Weg eingeschlagen, um die besagte Wechselbeziehung aufzuzeigen. Er arbeitete mit einer von Zimmer 1930 auf der Abderhalden'schen Reaktion begründeten Technik zur Bestimmung der spezifischen Abbaufemente des Blutes, um ein Bild von den endokrinen Funktionen bei Polyarthrititis zu gewinnen. Er machte in 160 Fällen interferometrische Bestimmungen und fand in fast sämtlichen Fällen Abweichungen von den normalen Abbaukurven der inkretorischen Organe — namentlich der Ovarien. Nach Sundelin's Auffassung haben die Versuche gezeigt, »dass die Beziehungen zwischen dem endokrinen System und der chronischen Polyarthrititis enger und mannigfaltiger Art und von ausserordentlicher Bedeutung für das Verständnis der Pathogenese der chronischen Polyarthrititis sind.«

An der Orthopädischen Klinik zu Lund, deren Chef bis Aug. 1940, Frising, stets ein lebendiges Interesse für die Beziehungen zwischen dem Knochengelenksystem und dem endokrinen System bekundet hat, wurde in den Jahren 1936—1940 eine Reihe von Bestimmungen der Hormonausscheidung mit dem Harn an den Patientinnen der Klinik, hauptsächlich chronischen Polyarthritiden, vorgenommen. Da wir also über ein verhältnismässig grosses Untersuchungsgut verfügen, halte ich es für gerechtfertigt, diese Daten zusammenzustellen. Da es gleichzeitig Interesse hätte, an demselben Untersuchungsgut auch andere Beziehungen zwischen Geschlechtsfunktion und Gelenkleiden zu beleuchten — grössere einschlägige Versuchsreihen sind nicht vorhanden —, ist 1943 eine Nachuntersuchung vorgenommen worden, und soweit es möglich war, haben wir den betreffenden Patientinnen Fragebogen zuge-

schickt, um ergänzende Aufschlüsse zu den in dieser Beziehung durchweg sehr knappen Angaben der Krankenblätter zu erhalten. Aus bestimmten Gründen war es notwendig, mit einem Kontrollgut zu arbeiten, dessen Nachuntersuchung jedoch in unserem Zusammenhang ohne Belang war und daher unterblieben ist. Nachdem Fälle mit zweifelhafter Diagnose usw. ausgeschlossen worden sind, fusst die vorliegende Untersuchung auf folgendem Material:

<i>Polyarthritiseriehe</i>	116 Fälle
<i>Kontrollreihe</i>	43 »

In der Polyarthritiseriehe ist in 102 Fällen der *Prolantiter* bestimmt worden, der *Follikulintiter* allein in 14 Fällen, in 3 Fällen beide Titer. In der Kontrollreihe ist nur der *Prolantiter* bestimmt worden. Die Nachuntersuchung umfasst nur 109 Patientinnen der Versuchsreihe. Von diese waren 4 inzwischen verstorben, von 3 war keine Antwort zu erhalten, weshalb dieser Teil der Untersuchung 102 Fälle umfasst, d. i. 90 % der ganzen Reihe. Diese 102 Patientinnen haben — in der Regel sehr ausführlich und gewissenhaft — den ihnen zugeschickten Fragebogen beantwortet.

Punkt I des Fragebogens betraf den Zivilstand mit Angabe des Datums der Eheschliessung usw. Punkt II erfragte die normalen Verhältnisse der Menstruation sowie ihre Beziehung zum Gelenkleiden. Entsprechend wurden unter Punkt III Angaben über die Schwangerschaften erhoben, mit Geburtsdaten der Kinder, Fehlgeburten u. dgl. Schliesslich wurden unter Punkt IV verschiedene Fragen über die Beziehung des Gelenkleidens zu Menstruation, Schwangerschaft und Menopause gestellt, und zwar so, dass sie die unter I—III gemachten Angaben kontrollieren und ergänzen konnten.

Sämtliche Hormonbestimmungen sind am hiesigen Hormonlaboratorium ausgeführt worden, dessen damaliger Vorstand, Doz. Med. Dr. Sune Genell, mir damals und auch jetzt bei der Bearbeitung des Materials durch die Kontrolle der Primärwerte und wertvolle Ratschläge geholfen hat, wofür ich ihm sehr zu Dank verpflichtet bin. Dass die Prolanbestimmungen in unserm Untersuchungsgut so stark überwiegen, erklärt sich damit, dass die damalige Technik keine genügende Genauigkeit der Follikulinbestimmungen bei den niedrigen Werten gestattete. Der *Prolantiter* wurde nach folgendem Verfahren ermittelt: Ausfällung einer gewünschten Menge angesäuerten Harns mit 95 %igem Alkohol, Zentrifugieren und Nachwäsche des Sediments, das nach Verdunstung in dest. Wasser gelöst wird. Das Konzentrat wird infantilen 3 Wochen alten Mäuseweibchen von 6—8 g Gewicht eingespritzt, und zwar in 6 Portionen, mit einem Intervall von 48 Stunden zwischen der ersten und der sechsten Injektion. Ab-

lesung 100 Stunden nach der ersten Einspritzung. Der Prolantiter betrifft in allen Fällen das sog. Prolan A.

Die »chronische Polyarthrit« ist ein schwer zu definierender Krankheitsbegriff. In der vorliegenden Arbeit wird unter dieser Diagnose das verstanden, was im deutschen Schrifttum als »primärchronische rheumatische Infektarthritis« und in der angelsächsischen Terminologie als »rheumatoid arthritis« oder »atrophic arthritis« bezeichnet wird. In der Polyarthritidenreihe finden sich 108 derartige Fälle, während in 8 Fällen die Gelenkbeschwerden hauptsächlich subjektiv und mehr vom Typus der »menopaus arthralgia« waren. Es erschien jedoch wertvoll, auch diese Gruppe bei einer Untersuchung der vorliegenden Art einzubeziehen. In der Zeit, welche die Untersuchung umfasst, sind an unserer Klinik 254 Patienten unter der Diagnose »Polyarthrit« chronica« versorgt worden. Die hier behandelten Fälle machen also 46 % des gesamten Polyarthritidengutes der Klinik aus. Es ist nicht ganz ausgeschlossen — namentlich gilt dies für die erste Zeit —, dass eine gewisse Auswahl getroffen worden ist. Vermutlich wird man häufiger in solchen Fällen die Titration ausgeführt haben, in denen klinische Gründe einen positiven Befund erwarten liessen. Das hier vorgelegte Material erweist sich jedoch in wichtigen klinischen Beziehungen als ein *typisches durchschnittliches Polyarthritidengut*.

Das *Durchschnittsalter* zur Zeit der Nachuntersuchung war 39 ± 0.9 Jahre. Fig. 1 und Tab. 1 zeigen die Altersverteilung und veranschaulichen die im ganzen gleichmässige Häufigkeitssteigerung mit zunehmendem Alter. Der steile Kurvenabfall nach dem 50. Lebensjahr ist teils darauf zurückzuführen, dass oberhalb dieser Altersgrenze seltener Titrationen gemacht wurden, teils und vor allem darauf, dass sich das Untersuchungsgut hauptsächlich aus Patientinnen zusammensetzt, die durch die staatliche Pensionsbehörde eingewiesen waren, und in der Regel liegt die Altersgrenze für die betreffenden Patientinnen der Lunder Klinik beim 50. Lebensjahr.

Von grösstem Interesse ist das *Alter bei Krankheitsbeginn*. Wir verfügen diesbezüglich über ein sehr wertvolles Vergleichsmaterial, da Edström (1936) eine entsprechende Zusammenstellung des Krankengutes der Medizinischen und Pädiatrischen Klinik zu Lund vorgenommen hat. Die Zusammensetzung unseres und des Edström'schen Materials wird weitgehend übereinstimmen, da die betreffenden Kliniken alle etwa denselben Aufnahmebereich und damit eine einheitliche Klientel haben. In unserem Material ist zwangsläufig die Verteilung infolge der durch die obengenannten Aufnahmebestimmungen bedingten Auswahl schief. Es zeigt sich (siehe Fig. 2 und Tab. 2), dass die Übereinstimmung bis zu der

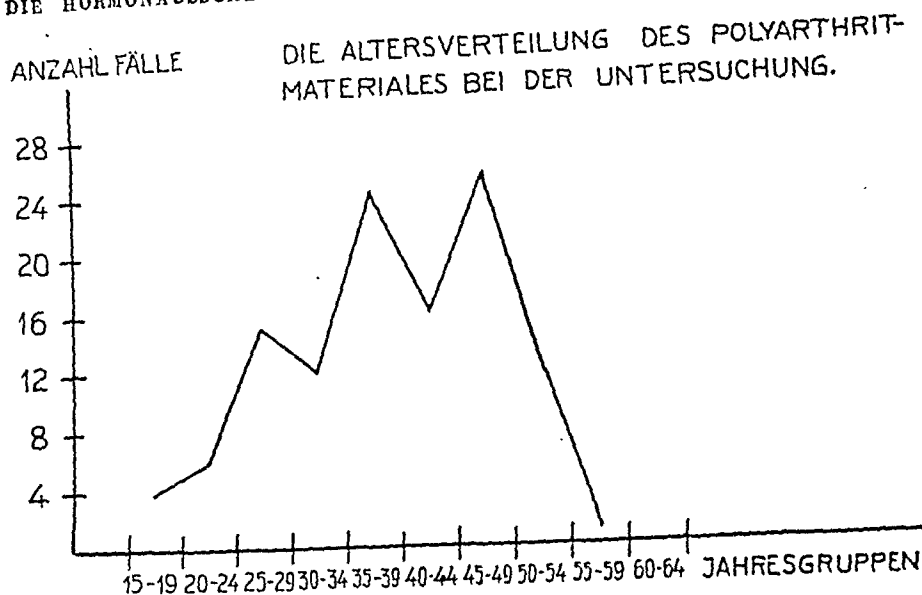


Abb. 1.

Tabelle I.

Die Altersverteilung und Heiratsfrequenz beim Krankenhauseintritt.

Alter (Jahre)	15—19	20—24	25—29	30—35	35—39	40—44	45—49	50—54	55—59	Zus.
Anzahl	4	6	15	12	24	16	25	12	2	116
Berechnete Heiratsfrequenz	0.08	1.6	8.9	8.5	17.3	11.4	17.3	7.8	1.2	74.1
Gefundene Heiratsfrequenz	0	1	9	8	12	12	19	10	1	72

Tabelle II.

Die Altersverteilung zur Zeit des Krankenhauseintritts.

Alter (Jahre)	0—2	3—5	6—10	11—15	16—20	21—25	26—30	31—35	36—40	41—45	46—50	51—55	56—60
Nach Edström	—	1	4	13	25	37	29	47	54	37	42	41	32
Eigenes Material	1	—	1	3	11	8	19	22	10	14	15	2	—

in Rede stehenden Altersklasse sehr gut ist. In beiden Materialreihen zeigen die Kurven deutlich eine gewisse Dreigipfligkeit; in dem Edström'schen Material sind diese Gipfel etwa ein Jahr fünf im Verhältnis zu unserm Krankengut verschoben. Wir werden hierauf noch zurückkommen. Hier soll nur festgestellt werden, dass die übereinstimmende Kurvenform

DIE ALTERSVERTEILUNG BEI KRANKENNAHME.

ANZAHL FÄLLE

EIGENE · NACH EDSTRÖM

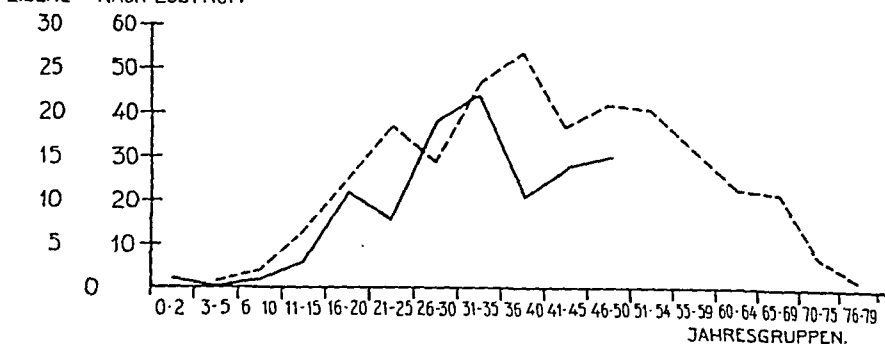


Abb. 2.

zu zeigen scheint, dass eine etwaige Auswahl der Titrationsfälle auf die Zusammensetzung in dieser Hinsicht keine merkliche Einwirkung ausgeübt hat.

Typus und Ausbreitung der Gelenkveränderungen waren die üblichen. Was die Ausbreitung betrifft, enthält das Material Fälle, in denen bloss einige Fingergelenke angegriffen waren, bis hin zu solchen, bei denen praktisch sämtliche Gelenke des Körpers von der Krankheit befallen waren.

Tabelle III.

Das Verhalten der Blutkörperchensenkungsgeschwindigkeit (SR). Erste Werte beim Krankenhauseintritt.

SR mm/Stde.	Polyarthritidenreihe		Kontrollenreihe
	Sämtliche Fälle	Ovarialinsuffizienz	
1— 10	10	1	16
11— 20	31	6	10
21— 30	20	2	7
31— 40	19	2	3
41— 50	9	1	1
51— 60	7	2	2
61— 70	4	—	—
71— 80	8	—	—
81— 90	3	—	—
91— 100	4	—	—
101—110	—	—	—
111—120	1	—	—

Die Ausbreitung der Veränderungen ist tabellarisch bearbeitet worden; da sich kein Prädilektionstypus der Gelenkveränderungen hat feststellen lassen, erübrigt sich eine Veröffentlichung dieser Bearbeitung.

Das Verhalten der Blutsenkungsgeschwindigkeit (SR mm/1 Stde.) ist in Tab. 3 veranschaulicht. Wie man sieht, besteht in der Polyarthritidenreihe gewöhnlich eine mässige Beschleunigung, während erwartungsgemäss die Kontrollen einen höheren Hundertsatz normaler Werte aufweisen.

Geschlechtsleben und Gelenkkrankheit.

Dieser Teil der Untersuchung ist das Ergebnis der Nachuntersuchung und hat die Aufgabe, die *subjektive* Auffassung der Patientinnen hinsichtlich des Zusammenhanges zwischen der Geschlechtsfunktion in casu und dem Gelenkleiden zu beleuchten.

Was die *Menstruation* angeht, braucht hier die bekannte Tatsache nicht näher erörtert zu werden, dass in dieser Weise gesammelte Angaben stets ziemlich unzuverlässig sind und daher nur einen beschränkten Wert haben. 94 Frauen haben Angaben über die *Menarche* gemacht, aus denen sich eine typische Kurve mit dem Höhepunkt im 15. Lebensjahr ergibt; die Verteilung ist die für unsere Klientel zu erwartende, eine sichere Verschiebung lässt sich nicht erkennen. Die eigenen Angaben über die Länge des Menstruationszyklus weisen in den Fällen, in denen die Krankenblätter eine Kontrolle erlaubten, so grosse Abweichungen von der tatsächlichen Länge auf, dass es sich kaum verlohnt, sie hier wiederzugeben. Grösseren Wert haben dagegen die Angaben über *Veränderungen im Charakter der Menstruation*, die von einem Teil der Patientinnen gemacht worden sind. So geben 6 Frauen häufigere Menstruationen und 17 Frauen längere Intervalle seit dem Auftreten der Krankheit an. Die subjektiven Menstruationsbeschwerden wurden in 5 Fällen stärker, in 2 Fällen geringer, ihre Menge wird in 13 Fällen als gesteigert, in 9 Fällen als vermindert angegeben. Insgesamt waren in 32 Fällen — 32 % des Materials — irgendwelche Störungen der Regel zu verzeichnen, während 52 Frauen keine Beeinflussung der Menstruation durch die Gelenkkrankheit bemerkt haben; in 18 Fällen sind die diesbezüglichen Angaben unsicher oder die Patientinnen haben nach der Erkrankung keine Menstruation gehabt.

Auf die Fragen nach dem *Verhalten der Gelenkkrankheit bei der Menstruation* haben 77 Patientinnen gute Antworten geliefert; in 25 Fällen fehlen diesbezügliche Angaben, oder die Patientinnen

haben keine Menstruation gehabt. 29 Patientinnen — 48 % derjenigen, welche die Fragen beantwortet haben, 28 % des Materials — melden eine entschiedene *Verstärkung der subjektiven Gelenkbeschwerden* während der Regel, nur 3 haben eine Linderung vermerkt; 45 konnten keine Änderung der Beschwerden feststellen. Häufig steigern sich also die Schmerzen in polyarthritisch erkrankten Gelenken während der Menstruation, und zwar besonders während der ersten Tage. Eine subjektive Linderung dagegen ist selten. Die Frequenz der veränderten Gelenkbeschwerden bzw. Menstruationsverhältnisse ist m. E. in dem vorliegenden Material von einer Grössenordnung, dass man einen Zusammenhang zwischen Geschlechtszyklus und Gelenkbeschwerden oder umgekehrt ahnen kann.

Das grösste Interesse beansprucht indessen das Verhalten der Gelenkkrankheit bei der *Schwangerschaft*. Zweifellos hatte man vielfach schon früher — so jedenfalls an der Orthopädischen Klinik — einen solchen Zusammenhang bemerkt, doch wurde er im Schrifttum erstmalig von De Sa (1937) hervorgehoben; später haben u. a. Touw et Knipers (1938) sowie Hensch (1939) darüber gehandelt. Um den rechten Hintergrund zu gewinnen, erscheint es notwendig, unser Material vom bevölkerungsstatistischen Gesichtspunkt aus zu beleuchten.¹ Selbstverständlich ist es sehr wertvoll, Faktoren wie *Heiratsfrequenz*, *Kinderzahl* usw. zu analysieren. Solche Daten dürfen als Indikatoren — im allerweitesten Sinne — dafür gelten, was man die *exkretorische Funktion* des Eierstocks nennen könnte, und ferner sind sie auch ein Ausdruck der Harmonie und des Gleichgewichts im komplizierten hormonalen Zusammenspiel des weiblichen Geschlechtszyklus.

Tab. 1 verzeichnet neben dem Alter auch die berechnete und die tatsächlich gefundene *Heiratsfrequenz* in den einzelnen Altersgruppen; die berechneten Werte stützen sich auf die schwedische Volkszählung vom Jahre 1940. Es zeigt sich, dass die Heiratsfrequenz der Polyarthritidenreihe so gut wie exakt der berechneten entspricht. In dieser Hinsicht repräsentiert das Untersuchungsgut also einen typischen Bevölkerungsdurchschnitt, und das Gelenkleiden hat die Heiratsaussichten nicht — wie man vielleicht hätte vermuten können — beeinträchtigt.

¹ Für wertvolle Hilfe bei dieser Analyse sage ich dem Statistiker der Universität Lund, Herrn Prof. Fil. Dr. C. E. Quensel, herzlichen Dank.

Tabelle IV.

Der Zusammenhang der Schwangerschaften mit dem Krankheitsbeginn (sämtl. Fälle).

(samt. 1. Aufl.)

Alter bei der Erkrankung:	Schwangerschaften vor dem Krankheitsbeginn						Schwangerschaften nach dem Krankheitsbeginn					
	5	5—4	4—3	3—2	2—1	1—0	0—1	1—2	2—3	3—4	4—5	5
15—20 J. ..	—	—	—	—	—	—	—	—	—	—	—	7
20—25 J. ..	—	—	—	—	—	1	—	—	—	—	—	2
25—30 J. ..	10	2	2	2	2	7	1	—	1	1	—	4
30—35 J. ..	14	2	5	2	4	5	1	—	1	1	1	5
35—40 J. ..	15	—	—	—	—	—	—	—	—	—	—	—
40—45 J. ..	30	—	—	2	—	—	—	1	1	—	—	1
45—50 J. ..	21	—	—	—	—	—	—	—	—	—	—	—
50—55 J. ..	9	—	—	—	—	—	—	—	—	—	—	—
	99	4	7	6	6	13	2	1	3	2	1	19
	135						28					

Über die *Kinderzahl* sind exakte Angaben nur durch den Fragebogen erhalten worden. Von den 102 Frauen der Polyarthritidenreihe waren 68 verheiratet, doch haben nur 60 vollständige Angaben gemacht; diese 60 Frauen hatten bis zum Zeitpunkt der Nachuntersuchung 153 Kinder, 13 Frauen waren kinderlos. Indessen hat das grösste Interesse für diese Frage der Zeitpunkt des Krankheitsbeginns. Bei Krankheitsbeginn waren 51 Frauen verheiratet und hatten zusammen 125 Kinder, während 10 kinderlos waren. Da Angaben über das Jahr der Eheschliessung und die Dauer der Ehe vorliegen, ist es möglich, die gefundene Kinderzahl und die Anzahl der kinderlosen Ehen mit den entsprechenden Verhältnissen der schwedischen Gesamtbevölkerung zu vergleichen. Geht man von den Erhebungen der Volkszählung vom Jahre 1936 aus, so müssten die 51 bei Krankheitsbeginn verheirateten Frauen 105 Kinder haben und die Zahl der kinderlosen Ehen 11 betragen. Die tatsächlichen Zahlen waren aber 125 bzw. 10. Bei der Nachuntersuchung hätten entsprechend die 60 Frauen mit Kindern insgesamt 175 Kinder haben müssen, so dass also theoretisch nach der Erkrankung 70 Kinder hätten geboren werden müssen. Tatsächlich sind nur 28, also ein geringer Teil der berechneten, geboren worden. Die Anzahl der kinderlosen Ehen hätte von 11 auf 8 fallen sollen, hat sich aber — nach Schliessung weiterer 9 Ehen —

vielmehr auf 13 erhöht. Allzu grosse Bedeutung in statistischer Hinsicht darf man wegen des kleinen Materials und der Schwierigkeit, exakte Vergleichsdaten zu finden, diesen Zahlen nicht beimessen, doch lassen sie erkennen, dass die Geburtenzahl besonders in den ersten Jahren der Krankheit (siehe Tab. 4) bedeutend kleiner ist, als man rechnerisch hätte erwarten können. Dies sind ausserordentlich interessante Daten. Die vor Krankheitsbeginn in dieser Gruppe von Frauen völlig normale Fertilität sinkt nach Ausbruch des Gelenkleidens katastrophal ab. Inwieweit die Erklärung dafür in allgemeinmenschlichen Faktoren, langem Krankenhausaufenthalt usw. oder in einer etwaigen Störung der Ovarialfunktion im Zusammenhang mit der Gelenkkrankheit zu suchen ist, lässt sich selbstverständlich nicht entscheiden.

Wie aus dieser Untersuchung ersichtlich ist, haben wir trotz des Umfangs des Materials nur recht wenige Fälle — im ganzen 17 — wo die Frauen nach ihre Erkrankung Kinder bekommen haben. Von diesen 17 Frauen haben 9 — also über die Hälfte — während der Schwangerschaft eine unverkennbare Linderung ihrer Beschwerden festgestellt. 3 Frauen melden umgekehrt eine Steigerung ihrer Beschwerden während der Gravidität, und nur eine hat deutlich angegeben, sie habe keinen Einfluss der Schwangerschaft auf die Gelenkkrankheit merken können. Die restlichen 3 Fälle machen in dieser Hinsicht unbestimmte Angaben, die keine sichere Bewertung ermöglichen. Nach dem Partus sind indessen die Gelenkbeschwerden nach kürzestens 4 Wochen und längstens 4 Monaten zurückgekehrt. In sämtlichen Fällen beurteilten die betreffenden Patientinnen diese erneuten Beschwerden als im Vergleich zu früher erschwert. In einem Falle (mit zwei Schwangerschaften) glaubte die Frau nach 1 Jahr sich gegenüber ihrem Zustand vor der ersten Schwangerschaft gebessert, doch bekam sie nach der zweiten Gravidität erneut starke Beschwerden. Auch hier können jedoch ausser den hormonalen Faktoren viele andere mitspielen, z. B. der Einfluss der gesteigerten Beanspruchung der Gelenke.

Wie weiter oben schon erwähnt, zeigt die Kurve des Erkrankungsalters eine gewisse Dreigipfligkeit, und zwar fällt in unserm Material der erste Gipfel in die Altersgruppe 16—18 Jahre, der zweite in die Gruppe 26—35 Jahre, der dritte, flachere in die Gruppe 41—50 Jahre; Edström's Material ergibt eine ebenso drei-

gipflige Kurve, doch sind die Gipfel im Verhältnis zu unserer Kurve etwa um 5 Jahre verschoben. Selbstverständlich geht es nicht an, aus einer solchen Kurve zu viel herauslesen zu wollen, doch legt die Tatsache als solche die Annahme eines Zusammenhanges zwischen dem Krankheitsbeginn und den 3 grossen Perioden des weiblichen Organismus — Pubertät, Fertilität und Klimakterium — nahe. Es ist u. a. deshalb von Interesse zu untersuchen, inwieweit wir aus den vorliegenden Angaben einen Zusammenhang zwischen der Erkrankung und Menarche, Schwangerschaft und Menopause herauslesen können. In 3 Fällen geben die Patientinnen selbst an, dass die Gelenksbeschwerden zugleich mit den Monatsregeln begonnen hätten, und aus den Antworten ist zu entnehmen, dass für die Patientinnen selbst kein Zweifel an dem Zusammenhang der beiden Ereignisse bestehen kann. In Tab. 4 ist unverkennbar eine Häufung von Schwangerschaften im Jahre des Krankheitsbeginns festzustellen. In 12 Fällen besteht auch ein klarer zeitlicher Zusammenhang zwischen Schwangerschaft (in zwei Fällen durch Abort beendet, wo also ein infektiöses Moment nicht ausgeschlossen werden kann) und Beginn des Gelenkleidens. Hinsichtlich der Menopause ist es nicht ebenso leicht, diese exakt zeitlich zu bestimmen. In 8 Fällen war jedoch die Gelenkkrankheit in zeitlich nahem Zusammenhang mit dem Aufhören der Blutungen aufgetreten.

In 23 der 102 nachuntersuchten Fälle lässt sich also ein zeitlicher Zusammenhang zwischen der polyarthritischen Erkrankung und hormonalen Stürmen der weiblichen Geschlechtsfunktion erkennen. Von Interesse ist die Feststellung, dass von den übrigen 79 Patientinnen 30 ein Zusammenfallen des Beginns der Polyarthrits mit einem entzündlichen Zustand (Angina, Erkältung, Scharlach usw.) melden. In der vorliegenden Polyarthritidenreihe sind diese beiden als ätiologische Momente besonders lebhaft erörterten Faktoren in etwa derselben Frequenz vorgekommen (in 30 % zeitlicher Zusammenhang mit einer Infektion, in 23 % mit der Geschlechtsfunktion).

Die durch die Nachuntersuchung erhaltenen Angaben lassen m. E. eindeutig erkennen, dass in einer nicht kleinen Anzahl von Fällen ein offensichtlicher Zusammenhang zwischen der weiblichen Geschlechtsfunktion und der chronischen Polyarthrits besteht.

Hormonausscheidung und Gelenkkrankheit.

Als zweite Hauptaufgabe dieser Arbeit stellt sich die Untersuchung dar, ob eine Beobachtung der Hormonausscheidung bei Polyarthritiden zu einer vertieften Analyse der offenbar sehr komplexen Relationen, mit denen wir es hier zu tun haben, beitragen kann. Der Prolantiter im Harn ist in 105 Fällen bestimmt worden, die Follikulinmenge wegen der damals vorhandenen technischen Schwierigkeiten in nur 14 Fällen. Das umgekehrte Verhältnis zwischen den Bestimmungen wäre vorteilhafter gewesen, da ja die Follikulinbestimmung einen direkteren Ausdruck für die Ovarialfunktion liefert, während die Prolanausscheidung nur sozusagen auf einem Umweg die Störung derselben registriert. Ein anderer Nachteil liegt in der Schwierigkeit, die Ergebnisse der Prolantitrationen richtig zu bewerten. Wir wissen nämlich noch nichts Genaues über den Verlauf der Prolankurve während des Geschlechtszyklus. Während wir betreffs der Follikulinausscheidung eine auf grossen Materialzusammenstellungen fussende Kenntnis von den Einzelheiten der Ausscheidungskurve besitzen (Genell 1943), fehlen entsprechende Angaben für die Prolantitration; die von Zondek angegebenen Werte sind zu summarisch, als dass sie eine wesentliche Hilfe böten. Es erschien daher notwendig, ein eigenes Kontrollmaterial für direkte Vergleiche mit der Versuchsreihe zu beschaffen.

Das Kontrollmaterial besteht aus 43 Frauen, die sich dem Alter nach etwa wie die Polyarthritidenreihe verhalten. Technisch haben Kontrollen und Gelenkkrankheiten die gleiche Behandlung erfahren. Die Diagnosen in der Kontrollenreihe lauten in der Hauptsache auf Insufficiencia dorsi oder Syndroma lymbo-ischias — etwa 35 % — doch finden sich auch 7 Fälle von Omarthritis und ein Fall von Spondylarthritis ankylopoetica darunter, welch letzterer Fall als besonders wertvoll angesehen wurde. Ferner lautete in 3 Fällen die Diagnose auf Adipositas, die übrigen Fälle boten ein buntes Bild mit den üblichen orthopädischen Diagnosen. Zur Blutkörperchengeschwindigkeit siehe Tab. 3.

Die Ergebnisse der Prolantitration sind aus den Tabellen 4—5 ersichtlich.

Zur Aufstellung der Tabellen sei nur bemerkt, dass die Spalte 40—80 ME auch diejenigen Fälle enthält, in denen die Titration nicht bis zu einer niedrigeren Grenze fortgesetzt worden ist, sondern bei denen die Antwort nur »weniger als 80 ME« lauten konnte; es ist also nicht undenk-

Tabelle V.

Die Prolanausscheidung in verschiedenen Altersklassen.

Alter	Prolantiter in ME				
	bis 40 ME	40—80 ME	80—100 ME	100—165 ME	165 ME
<i>I. Polyarthritiden.</i>					
15—19 J.	2				
20—24 J.	—	2	2		
25—29 J.	7	4	2	1	
30—34 J.	6	4	1	—	
35—39 J.	8	5	2	1	2
40—44 J.	10	3	—	3	—
45—49 J.	9	4	—	4	7
50—54 J.	—	—	—	2	10
55—59 J.	—	—	—	—	2
<i>II. Kontrollen.</i>					
15—19 J.	2	1			
20—24 J.	1	4			
25—29 J.	3	2			
30—34 J.	—	3			
35—39 J.	4	2	1		
40—44 J.	4	1	—	1	
45—49 J.	3	1	—	2	1
50—54 J.	—	1	—	—	2
55—59 J.	—	—	—	1	1
60—64 J.	—	—	—	—	1
65—69 J.	—	—	—	—	1

bar, dass ein Teil dieser Fälle eigentlich zur nächsttieferen Gruppe gehört hätte, falls nur die Bestimmung weiter nach unten fortgesetzt werden wäre.

Die Tabellen zeigen das bekannte Bild — in den Jahren der Geschlechtsreife niedrige Titerwerte, die bei der Menopause schnell ansteigen; jenseits des 50. Lebensjahrs hat in der Versuchsreihe keine, in der Kontrollenreihe eine Patientin Werte unter 100 ME. Die beiden Reihen weisen frappante Ungleichheiten auf. Während unter den Polyarthritiden die höheren Gruppen auch bei Frauen jüngeren Alters vertreten sind, fehlen solche Titerwerte bei den Kontrollen fast ganz. Dies deckt sich mit der Erfahrung, dass Werte über 80 ME mit einer normalen Ovarialfunktion nicht vereinbar sind, sondern dass sie erst auftreten, wenn

Tabelle VI.

Die Prolanausscheidung in verschiedenen Altersklassen (Zusammenfassung).

Alter	Prolanausscheidung in ME				
	bis 40 ME	40—80 ME	80—100 ME	100—165 ME	165 ME
<i>I. Polyarthritiden.</i>					
15—39 J.	23	15	7	2	2
	47 %	31 %	14 %	4 %	4 %
40—49 J.	19	7	—	7	7
	48 %	17 %	—	17 %	17 %
50— J.	—	—	—	2	12
				12 %	88 %
<i>II. Kontrollen.</i>					
15—39 J.	10	12	1	—	—
	44 %	52 %	4 %	—	—
40—49 J.	7	2	—	3	1
	54 %	15 %	—	23 %	8 %
50— J.	—	1	—	1	5
	—	14 %	—	14 %	72 %

die Follikulinproduktion pathologisch niedrige Werte erreicht hat. Es ist möglich (laut persönlicher Mitteilung von Genell), dass die Grenze vielleicht noch tiefer angesetzt werden könnte, vielleicht bei 65 ME, da aber die Titrationsen bei unseren Fällen hauptsächlich in den in der Tabelle angegebenen Grenzen blieben, sowie um die Grenze nicht zu eng zu ziehen, empfiehlt es sich, Werte von 80 ME und höher als mit einer normalen Ovarialfunktion unvereinbar festzusetzen. Im Klimakterium und in der Menopause treten solche Werte normal auf, vor diesem Alter aber sind sie krankhaft. In der Polyarthritidenreihe finden wir, dass von 49 Frauen unter 40 Jahren, vor welchem Alter das Klimakterium normalerweise nicht vorkommt, 11 zu hohe Prolantiterwerte hatten. Bei den Kontrollen findet sich in derselben Altersgruppe nur 1 Fall. Von den Polyarthritiden zwischen 15 und 39 Jahren zeigen 22 % einwandfrei pathologische Werte, während bei den Kontrollen derselben Altersgruppe nur 4 % einen erhöhten Prolantiter aufweisen. Die Differenz ist 18 ± 7.2 %, somit reichlich doppelt, jedoch nicht ganz 3mal so gross wie der mittlere Fehler, weshalb sie statistisch wahrscheinlich ist.

Die Anzahl der Follikulintitrationsen ist zu gering, als dass man

sie mit irgendwelchem Nutzen entsprechend wie die Prolanwerte bearbeiten könnte; hier haben wir jedoch die Möglichkeit eines direkten Vergleichs mit bekannten Normalwerten. Von 14 Bestimmungen entfallen 12 auf die Altersgruppen 5—39 Jahre, 2 auf die Gruppe 40—49 Jahre. Bei den ersteren ergaben sich in 2 Fällen einwandfrei subnormale Werte, ein dritter Wert steht auf der Grenze zum Abnormen.

An einem Krankengut von 61 Frauen unter 40 Jahren mit chronischer Polyarthritis haben wir also durch exakte Hormonbestimmungen in 13 Fällen eine Ovarialinsuffizienz feststellen können. Ein Kontrollmaterial mit verschiedenen orthopädischen Diagnosen, das Frauen derselben Altersgruppen umfasste, wies dagegen nur einen solchen Fall auf.

Die betreffenden 13 Fälle sind einer eingehenderen Prüfung unterzogen worden, doch verraten sie hinsichtlich des Beginns, des Verlaufs oder des klinischen Typus der Krankheit (die SR-Werte siehe in Tab. 3) keine charakteristischen Züge, mit der einzigen Ausnahme, dass in ein paar Fällen merkbare Menstruationsstörungen vorlagen. Bei einer Frau in den Dreissigern hörten die Menstruationen mit dem Einsetzen der Gelenkbeschwerden auf und sind bisher — 4 Jahre später — noch nicht zurückgekehrt. Weitere 5 der 13 Frauen melden spärliche Menstruationen in langen Abständen, während die übrigen auf Grund der eigenen Angaben wie auch nach den Angaben der Krankenblätter als normal menstruierend rubriziert werden können. Es verdient erneut unterstrichen zu werden, dass sich der klinische Typus dieser Fälle in nichts von dem der übrigen Fälle unterscheidet.

Neben der Ovarialfunktion ist in dem vorliegenden Material auch ein anderes inkretorisches Organ untersucht worden, nämlich die Schilddrüse. Edström (1939) fand bei einem grossen Polyarthritidengut einen Grundumsatz, der im Durchschnitt ein wenig unter der Norm lag. Der mittlere Wert aus 65 Bestimmungen der Polyarthritidenreihe unseres Materials ist $+ 3 \%$; wenn man — wie Edström — $\pm 10 \%$ als Normalwert ansetzt, finden wir in unserem Material 10 % zu niedrige, 71 % normale und 20 % zu hohe Werte, in schöner Übereinstimmung mit Edström's entsprechenden Befunden: 14 %, 61 % und 25 %. Es erscheint recht wertvoll, in einem anderen inkretorischen Bereich aufzeigen zu können, dass unser Polyarthritidengut völlig mit den bisher bekannten Verhältnissen übereinstimmt.

Diskussion.

Wir haben also objektiv feststellen können, dass ein gutes Fünftel eines Materials von polyarthritischen Frauen unter 40 Jahren eine Ovarialinsuffizienz aufweist, sowie dass subjektiv ein klarer Zusammenhang zwischen der Gelenkkrankheit und der Geschlechtsfunktion, bzw. umgekehrt, in etwa einem Viertel der Fälle besteht. Indessen liegt keine positive Korrelation zwischen den Fällen dieser Gruppen vor, die sich anscheinend regellos über die ganze Serie verteilen. Es wäre aber von grossem Interesse, wenn sich ein innerer Zusammenhang zwischen diesen Befunden nachweisen liesse. Der gemeinsame Nenner, an den in erster Linie zu denken wäre, ist die *Follikulinwirkung*, der ja schon Rasmussen (1936) essentielle Bedeutung beigemessen hat. Ovarialinsuffizienz bewirkt verminderte Follikulinproduktion. Eine niedrige Follikulinproduktion ist — auch bei sonst normaler Ovarialfunktion — zur Zeit der Menstruation vorhanden, und subjektiv bestand auch fast in der Hälfte der Fälle an den betreffenden Tagen eine Verschlimmerung der Gelenkbeschwerden. Bei der Schwangerschaft ist die Follikulinproduktion stark erhöht — und in der Hälfte der Fälle, die nach Krankheitsbeginn eine Schwangerschaft gehabt haben, trat im Zusammenhang damit eine merkbare Linderung der Gelenkbeschwerden ein. Dies sind sehr interessante Tatsachen, die m. E. stark für die Richtigkeit der Rasmussen'schen Ansicht sprechen. Indessen gibt es auch starke Argumente gegen diese durch ihre Einfachheit ansprechende Deutung der Ätiologie der Polyarthritis. Die numerische Frequenz der Ovarialinsuffizienz ist in dem vorliegenden Material auch zu gering, als dass man sie als einen generellen Faktor betrachten könnte. Auch kann man, trotz gewisser Erfolge bei Follikulinmedikation — Kuipers (1935), Rasmussen (1936), Mall (1936), Murray (1941) —, nicht von einer erfolgreichen Substitutionstherapie bei der chronischen Polyarthritis sprechen. Die Frauen des vorliegenden Materials haben nur sporadisch Follikulin erhalten, und zwar, wie die Erfahrungen der letzten Jahre lehren, in viel zu kleinen Gaben, als dass man positive Resultate hätte erwarten können; es erübrigt sich daher, diese Resultate näher zu analysieren.

Ein Hauptergebnis der Untersuchung scheint es zu sein, dass keine Gründe bestehen dürften, die Fälle vom Typus der »meno-

paus arthralgia» aus dem generellen Polyarthritsbegriff herauszulösen, da die Untersuchung gezeigt hat, dass eine Ovarialinsuffizienz auch ausserhalb des Menopausenalters eine so häufige Erscheinung ist. Doch verfügen wir m. E. noch nicht über hinreichende Erkenntnisse für eine fruchtbare Fortsetzung der Diskussion. Dass wir es hier mit einem äusserst komplexen Geschehen mit einem engen, von Fall zu Fall wechselnden Zusammenspiel verschiedener Faktoren — exogener wie endogener —, über deren inneren Zusammenhang wir uns nicht äussern können, zu tun haben, ist wahrscheinlich. Der Zukunft und einer verfeinerten hormonalen und klinischen Analyse bleibt es vorbehalten, einen Weg durch dieses Labyrinth zu bahnen. Auf der Basis der hier gemachten Beobachtung ist es m. E. äusserst wahrscheinlich, dass das endokrine System einen hervorragenden Platz in der ätiologischen Diskussion einnehmen wird, mögen nun die Störungen dieses Systems sich als primäre Faktoren oder nur als Sekundärerkrankungen des Grundübels der chronischen Polyarthrits erweisen.

Zusammenfassung.

Die Untersuchung stützt sich auf ein in den Jahren 1936—40 zusammengebrachtes Material von Hormonanalysen an weiblichen Patienten mit chronischer Polyarthrits in der Orthopädischen Klinik zu Lund. Die Hormonanalysen werden ergänzt durch eine im Jahre 1943 durchgeführte schriftliche Nachuntersuchung. Das Material gliedert sich in zwei Reihen: 1) 116 Fälle mit chronischer Polyarthrits, 2) 43 Kontrollen mit wechselnden orthopädischen Diagnosen. An 61 polyarthritischen Frauen im Alter von 15—39 Jahren konnte die Hormonanalyse (Prolantitrationen in 49, Follikulinbestimmungen in 12 Fällen) in 13 Fällen eine sichere Ovarialinsuffizienz nachweisen, während unter den 23 Fällen des Kontrollmaterials nur einer eine solche Störung aufwies.

An demselben Krankengut wurden die subjektiven Beziehungen zwischen Geschlechtsfunktion und Gelenkkrankheit analysiert, wobei sich ein reiches Zusammenspiel feststellen liess. So waren Menstruationsstörungen nach der Erkrankung in 32 % der Fälle zu verzeichnen, in 48 % der Fälle konnte während der Menstruation eine deutliche Verschlimmerung der Gelenksbeschwerden bemerkt werden, während nur 4 % der Frauen eine Linderung der

Beschwerden melden. Hinsichtlich der Heiratsfrequenz und der Kinderzahl entspricht die Polyarthritidenreihe vor dem Auftreten der Krankheit einem Normalmaterial, während dagegen die Zahl der nach dem Ausbruch des Gelenkleidens geborenen Kinder stark beschränkt ist. Nur 17 Frauen (mit 28 Kindern) wurden nach Krankheitsbeginn schwanger. Von diesen melden 9 verminderte Gelenkbeschwerden, 3 verstärkte, 1 unveränderte, 4 geben unsichere Reaktionen an; in den Fällen mit während der Schwangerschaft leichteren Beschwerden verstärkten sich dieselben erneut 4 Wochen bis 4 Monate nach dem Partus. Bei fast einem Viertel der Fälle bestand ein zeitlicher Zusammenhang des Krankheitsbeginns mit Menarche, Schwangerschaft oder Menopause; in 30 % des Gesamtmaterials bestand ein zeitlicher Zusammenhang des Krankheitsbeginns mit einer Infektion.

In der Diskussion unterstreicht der Verfasser die Bedeutung der sicheren Feststellung dieser hohen Frequenz von Ovarialsuffizienz in einem Polyarthritidengut. Angesichts der hier erhobenen Befunde erscheint eine Abtrennung der Fälle vom Typus einer »menopaus arthralgia« unbegründet. Die Rolle des Follikulins als des vielleicht dominierenden Faktors wird erörtert. Der Verf. steht indessen auf dem Standpunkt, dass es sich hier um ein weit komplexeres Geschehen handelt. Dass endokrine Momente, primär oder sekundär, in der Pathogenese und Ätiologie der chronischen Polyarthrititis eine Hauptrolle spielen, ist nach der vorliegenden Untersuchung sehr wahrscheinlich.

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Studies on the causation of experimental gastroprival pellagra.¹

III. Therapeutic Experiments on Pups with Preventive Parenteral Administration of Vitamin B₆ — given together with Vitamin B₁, Lactoflavin and Nicotinic Acid Amid or Alone.

By

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(Submitted for publication January 5, 1944).

Introduction.

Our systematic studies on experimental endogenous gastroprival pellagra («the gastrogenous neuro-cutaneous symptom complex») were aimed primarily to elucidate the clinical and morphological aspects of the lesion, secondarily its etiology.

Attempts have been made to elucidate the etiology by means of various elective resections of the digestive tract together with therapeutic experiments.

The results of preventive parenteral treatment of totally gastrectomized pigs with nicotinic acid, vitamin B₁, lactoflavin and vitamin A, given alone or combined with vitamin B₁ + vitamin A have been reported by us (1938, 1940 and 1940). None of these

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Translation from Danish by Hans Andersen, M. D.

The vitamin B₆ preparation, which is not yet put on the market, was kindly placed at our disposal by the «Bayer» concern.

substances had any effect on the symptoms or course of the gastroprival pellagra.

A systematic extension of these therapeutic experiments would naturally aim to investigate the amenability of the pellagra to treatment with vitamin B₆ alone, various combinations of vitamins B and, finally, all the vitamins given simultaneously. In view of the negative outcome of the previous experiments and hoping to reach a conclusive solution of the problem more rapidly, we deviated from this plan and submitted totally gastrectomized animals at once to treatment with all the vitamins B (Group I). For various reasons we had to use pups instead of pigs for these experiments. Contrary to our expectations, for the first time in our therapeutic experiments we obtained a positive result in this way. In order to ascertain the part played by vitamin B₆ in this effect, therefore, it was necessary to perform an additional experiment with this substance alone (Group II). These two experimental groups will be reported in the following.

Material and Technique.

The experimental material comprises 4 pups, two sibs in each experimental group. The animals were of the same mongrel, short-haired and medium-sized, breed and of the same sex (female). At the commencement of the experiment the animals in Group I (Nos. 127 and 128) were 5—6 months old, weighed respectively 5.9 and 6.2 kg and measured 51 and 55 cm in length. In Group II (Nos. 131 and 132) the age was 4—5 months, the weight 5.8 and 5.0 kg, the length 49 and 48 cm. Our previous studies on the consequences of the same type of operation in pups have served as control (see pag. 11).

The operation was performed under ether anesthesia. The entire stomach was removed together with the proximal part (about 2 cm) of the duodenum, corresponding to the area where the Brunner glands are located. End-to-end anastomosis of the duodenum and oesophagus was performed after the method adopted by one of us (Petri).¹

¹ The anastomosis was performed, without employment of forceps, in the following stages: 1) distal division (of the duodenum); 2) extraction of the stomach which then is turned upwards, so that the oesophagus is pulled downwards and fixed suitably by the tightening; 3) suturation of the serosa posteriorly between the duodenum and the posterior wall of the distal part of the oesophagus; 4) transversal division of the posterior wall of the oesophagus just distally to the serosa suture, followed by 5) suturing of the mucosa posteriorly; 6) final division of the oesophagus (anterior wall) with 7) conclusive suturing of the mucosa and serosa anteriorly.

The diet has been the same as usually employed for dogs: squealer, coarse rye bread, sometimes buttered, sometimes larded; milk or milk-water; adequate addition of Cowgill's basal diet + yeast extract and cod liver oil (10 cm³ a week to each animal).

The experiments were commenced in September 1940 (Group I) and August 1941 (Group II).

The observation period has been 212 days (Group I) and 384 days (Group II). At the conclusion of the experiments the animals were killed by bleeding under ether anesthesia.

Vitamin Therapy. The following vitamin preparations and doses in cm³ per animal per week were employed, injected intramuscularly. *Group I:* Betaxin (Bayer) 1.75 cm³ (5 mg synthetic crystalline vitamin B₁ per cm³) + Lactoflavin (Hoffmann-La Roche) 7 cm³ (1 mg per 2.2 cm³) + nicotinic acid amid (Bayer) 0.5 cm³ (10 cg per 2 cm³) + vitamin B₆ (Bayer) 0.7 cm³, later 0.35 cm³ (originally 5 mg later from December 1940) 10 mg synthetic crystalline adermin hydrochloride per cm³. *Group II:* Vitamin B₁ alone; 1.5 cm³ in the first two months, 0.6 cm³ in the rest of the period (10 mg per cm³).

The vitamin preparations mentioned were given as $\frac{1}{3}$ weekly dose every other day (Sundays excluded). Regardless of minor differences in their weight, the animals in each group were given the same weekly dose from start to finish. The injections were given continually throughout the period of treatment, each preparation separately, in varying places. The period of treatment has been 202 days, commencing 10 days after the operation (Group I), and 384 days, from the day of operation (Group II). The prolongation of the period of treatment in Group II was aimed to eliminate the possibility of an exceptionally late appearance of gastropival pellagra and to render the possible effect of the treatment more convincing.

The total amounts of vitamins given each animal were: 25.1 cg of vitamin B₁ + 90.9 mg of lactoflavin + 72 cg of nicotinic acid amid + 10 cg of vitamin B₆ in Group I; and 40 cg of vitamin B₆ alone in Group II.

In Group I the daily dose per kg of body weight was: vitamin B₁, minimum 152—147 γ , maximum 236—223 γ ; lactoflavin, min. 55—53.4 γ , max. 85.7—81 γ ; nicotinic acid amid, min. 430—420 γ ; max. 675—635 γ ; and vitamin B₆, min. 60.6—58.8 γ , max. 94.3—89.1 γ . Group II: vitamin B₁, during the first two months, min. 343—376 γ , max. 392—438 γ ; in the remainder of the therapeutic period, min. 137—163 γ , max. 157—175 γ .

The vitamin doses were chosen on the basis of the following data: the minimum requirement of vitamin B₁ in infants is 240 γ (Cowgill); optimal growth requires 80 γ per kg per day (Knott and Poole *et al.*). In dogs lactoflavin avitaminosis is prevented by 25 γ of lactoflavin per kg per day; recovery requires a minimum intake of 750 γ per kg (Street & Cowgill). The minimal curative dose of nicotinic acid for experimental black-tongue in dogs is 0.5 mg per kg per day for 10 days (Margolis *et al.*). In nicotinic acid-treated pellagrous patients with refractory nervous symptoms, one injection of 50 mg synthetic adermin gives rapid and complete recovery (Spies, Bean & Ashe). In experimental B₆ deficiency in adult dogs a dose

of 60 γ per kg per day, given by mouth, is curative (Fouts, Helmer & Lepkovsky). The organism is said to have a great capacity for depositing of vitamin B₆.

According to the cited statements, in Group I the daily dose of vitamin B₁ has been a little lower than, or corresponding to, the minimum required; the daily dose of lactoflavin was 2—3 times the preventive dose; the daily dose of nicotinic acid amid was a little under or a little over the minimal curative dose; finally, the vitamin B₆ intake corresponded to the curative dose or was a little over.

In Group II the daily dose in the first 2 months was 5—7 times the curative dose, in the rest of the therapeutic period only a little over twice the curative dose.

Considering the parenteral administration of the drugs, the long period of treatment and the young age of the animals, the vitamin doses here employed may thus be characterized as rather large.

For the sake of comparison, it may be mentioned that the doses of vitamin B₁, lactoflavin and nicotinic acid in our previous therapeutic experiments on totally gastrectomized pigs on an average were respectively 5/4, 4 and 3—7 times larger than the doses here employed.

Experimental Results.

Group I.

Clinical Changes. — In both animals there developed a chronic, not fatal, morbid condition characterized by marked inhibition of growth, pronounced emaciation, minimal changes in the skin and hair and moderate changes in the blood picture. On the whole, both animals presented the same changes.

In one animal (No. 127) the weight remained almost unchanged during the first 2 $\frac{3}{4}$ months; during the following month the weight increased to its maximum (8.25 kg.). In the other animal (No. 128) the weight increased from the very start till it reached its maximum (8.5 kg). In both animals the maximal weight was reached at about the same time, 3 $\frac{1}{2}$ —4 months after the operation. After this the weight kept rather unchanged; the last weight was respectively 7.74 and 7.99 kg. The total increase in length amounted only to 7 and 3 cm, respectively. Apart from the first couple of months, the animals were rather emaciated throughout the experimental period.

The skin changes made their appearance after 4 months in the form of a single, small, thin-haired spot on the forehead with dry

scaling; the growth of hair on those areas that were shaved for the injections was somewhat more slow than normally.

At no point of time could changes in the central nervous system be noticed clinically; in particular the posture and gait were normal.

Changes in the blood: The hemoglobin percentage decreased rather gradually from 71 in both animals to 56 and 49, respectively. The red blood count decreased primarily from the initial values of 4.91 and 4.77 to 3.66 and 3.63 millions respectively; and 2—3 months later it rose again gradually, reaching finally 5.96 and 5.63 millions respectively. The color index was initially 0.72 and 0.74 respectively and terminally 0.47 and 0.44. The average diameter of the red blood cells decreased gradually from 7.08 and 6.73 μ to 6.23 and 5.9 μ terminally; the extreme values were reduced by 0.5 μ . The red blood count, which initially was respectively 7620 and 7360, showed a slight transitory tendency to rise (maximum respectively 9720 and 10480), but otherwise it was rather unchanged.

Also the general condition of the animals was subject to some variation. On the whole, however, the animals were lively and fond of being caressed. The appetite and defecation were normal except for a period of 1—2 weeks in the middle of the observation period, when the appetite was poor and diarrhea was present.

Morphological Changes. — The site of the anastomosis looked normal, presenting merely a small atrophic remnant of the gastric mucosa that could be demonstrated only microscopically. In one of the animals (No. 128) the proximal part of the duodenal mucosa showed considerable infiltration with lymphocytes and plasma cells. The organs showed no abnormalities, macroscopic or microscopic.

Bone-marrow of vertebrae: Moderate amount of fat cells. Femur: Fatty marrow, extremely poor in cells; with rather marked oedema (No. 127). Tibia, same findings as in the femur.

Central nervous system: No histological abnormality revealed by the method of examination usually employed in these experiments (cf. Nørgaard, 1942). No degenerative changes could be demonstrated in the nerve-cells and medullary sheaths, nor any reaction on the part of the glia — neither in the spinal cord, medulla oblongata, brain stem, cerebrum nor cerebellum.

Group II.

Clinical changes. — Both animals developed a chronic, non-fatal, morbid condition characterized by marked inhibition of growth and pronounced emaciation, slight changes in the skin and hair, and moderate changes in the blood picture. On the whole, the changes were alike in the two animals.

The weight decreased a little during the first month (to 5.46 and 4.89 kg respectively); in the following month it rose again to maximum (6.25 and 5.7 kg). During the next 8 months the weight kept unchanged, corresponding to the initial values. In the last 2 ½ months the weight decreased a little, being finally 4.94 and 4.48 kg respectively. The total increase in length amounted only to 5 cm in either animal. Apart from the first couple of months, the animals were rather emaciated throughout the experimental period.

The skin changes were characterized by the appearance of a small, thin-haired, later bare, area in the middle of the forehead; subsequently this process extended a little, and similar, symmetrical changes were observed round the eyes and on the sides of the nose. These changes did not appear till about 9 months (No. 131) and 10 months (No. 132) after the operation, and then they progressed as mentioned; the areas involved were scaling.

Central nervous system: At no point of time could any changes be noticed clinically; in particular, the posture and gait were normal.

Changes in the blood: The hemoglobin percentage was varying slightly between the initial value of 67 and 80, respectively and the minimum value of 52 and 47; the terminal value was respectively 56 and 52. The red blood count likewise showed moderate variations, differing a little in the two animals. In No. 131 the count varied between the initial 5.62 and the minimum value 4.31; the terminal value was 4.47. In No. 132, on the other hand, the red blood count varied from the initial 4.3 millions to the maximum 5.89 and the minimum 4.01; here the terminal count was 5.39 millions. The color index was initially 0.71 and 0.78; terminally 0.58 and 0.52. The average diameter of the red blood cells decreased rather gradually from 7.0 and 7.2 μ to 5.9 and 5.6 μ (about 2 ½ months.

after the operation), whereafter it increased gradually to 6.68 and 6.4 μ (terminally). The extreme values were reduced by 0.5–1.0 μ . The white blood count, which initially was 12000 and 12900, increased somewhat during the first 4 months to the maximum values of 20640 and 15320 respectively; subsequently it decreased again to the terminal values of 10400 and 11420.

As to the symptoms, the inhibition of growth and emaciation were rather stationary, whereas the hair changes were slightly progressive, and the blood changes slightly varying. The general condition was good throughout the experimental period, the animals being lively, watchful and fond of being caressed. The appetite and defecation were constantly normal.

Morphological Changes. — The site of the anastomosis appeared normal, with a slight atrophic remnant of gastric mucosa which could be demonstrated only microscopically. Sections from the proximal part of the duodenum showed considerable infiltration with lymphocytes and moderate infiltration with plasma cells in one of the animals (No. 132). The organs showed no macroscopic or microscopic changes.

Bone-marrow of vertebrae: Slight increase in the fat cells (+ slight oedema). Femur: Moderate (No. 131) and considerable (No. 132) amount of cells (+ slight oedema). Tibia: Fatty marrow, almost free from cells (+ pronounced oedema (No. 131) and minimal oedema (No. 132)].

Central nervous system: In No. 131 the sites of choice of the gastroprival changes in the central nervous system — the medulla oblongata and the brain stem at the level of the corpora quadrigemina, especially ventrally, among the medium-sized and small nerve-cells — showed some sclerotic cells and moderate microglia reaction, together with a few medium-sized nerve-cells undergoing changes as in »Nissl's primary irritation». In the cerebellum a few Purkinje cells were light-staining, swollen and vacuolized. The other examined parts of the central nervous system presented no abnormality. In No. 132 no abnormality was demonstrated.

Recapitulation.

In 4 pups the entire stomach + the proximal 2 cm of the duodenum (the Brunner gland area) was removed, whereafter the animals were given continuous preventive parenteral treatment

with vitamin B₁ + lactoflavin + nicotinic acid amid + vitamin B₆ (Group I) or with vitamin B₆ alone (Group II). The period of treatment was 202 days (Group I) and 384 days (Group II), the total observation period respectively 212 and 384 days. The total dose of vitamin B per animal was respectively 10 and 40 cg.

Clinically, in all the treated animals there developed a chronic non-fatal morbid condition characterized by inhibition of growth, emaciation, scanty and limited symmetrical changes in the skin and hair, together with the following changes in the blood picture: a moderate fall in hemoglobin percentage, varying red blood count (more often a little increased terminally) and microcytosis. The organs appeared normal, in particular no microscopic pathological changes in the digestive canal. The bone-marrow in Group II was slightly hyperplastic. The central nervous system showed slight degenerative changes in one animal (No. 131) but appeared normal in the remaining three animals.

Effect of the Vitamin Therapy on the Experimental Gastroprival Pellagra.

On comparison of the clinical picture presented by the above-mentioned pups with the features of untreated gastroprival pellagra in the same species as previously described by us, an expression is obtained for the therapeutic effect of the vitamins B employed on this morbid condition.

Gastroprival pellagra (see, for instance, Petri & Jensenius and Petri, Nørgaard & Jensenius) appears constantly after total gastrectomy in the form of a severe chronic lesion, terminating fatally within half a year, and characterized by: marked inhibition of growth («arrest of growth») and emaciation, symmetrical pronounced loss of hair, skin changes, pronounced clinical and very severe morphological changes in the central nervous system, changes in the blood picture (most often of the simple anemic type together with constant microcytosis), and hypoplasia (+ hyperemia and oedema) of the bone-marrow.

The clinical picture in the vitamin-treated, gastrectomized animals differs in several respects from that of untreated gastroprival pellagra. Thus, in the first-mentioned animals the lesion constantly



Fig. 1. Untreated gastropriaval pellagra (No. 53). (Petri, 1937). Observation period 6 months. Photo taken on the day before the death of the animal.

appeared in a much milder form; it was stationary or slightly varying, but not fatal (with an observation period of 7 and 12 $\frac{3}{4}$ months, respectively).

The skin and hair changes occurred only in a slight degree, limited exclusively to the initial localization characteristic of untreated gastropriaval pellagra (round the eyes, on the forehead and on the sides of the nose). The red blood count showed more often a slight terminal increase than a slight fall, whereas the hemoglobin percentage constantly was lowered moderately.

Clinical changes in the central nervous system did not occur, and microscopic changes were seen only in one animal (slight degree).

On the other hand, in spite of the treatment, the emaciation was rather marked, but not extreme; and the inhibition of growth, together with the microcytosis, was completely unaffected by the treatment. The cell content of the bone-marrow was normal in Group I, while the marrow was moderately hyperplastic in Group II; both groups showed a varying degree of oedema of the bone-marrow.

Thus preventive, parenteral, protracted treatment of totally gastrectomized pups with all the vitamins B₆ or with vitamin B alone has



Fig. 2. Gastropival pellagra treated with B_6 alone, preventively (intramuscular injection). No. 131 (= No. 132). Observation period $12\frac{3}{4}$ months. Period of treatment $12\frac{3}{4}$ months. Photo taken 5 days before the animal was killed.

exerted a pronounced effect on the experimental gastropival symptom complex, especially as far as the changes in the skin, hair and central nervous system are concerned.

The clinical features were the same in the individual animals within each group, but the two groups showed minor differences in the degree of the symptoms. Thus the treatment with all the vitamins B appears to have had a little more favorable effect as to the inhibition of growth, the skin and hair changes and the decrease in the red blood count; on the other hand, in this group the microcytosis was rather more pronounced (but with less dispersion of the values), and the general condition was more variable, associated with periodical diarrhea, than in the group treated with vitamin B_6 alone.

Morphologically the animals in Group II differed only with regard to the slight changes in the central nervous system; and the two experimental groups differed only in the features of the bone-marrow.

Comments.

Finally the question arises as to what contribution these experiments make to the elucidation of the causation of the experimental gastropival (endogenous) pellagra and the mode of action of vitamin B_6 in the organism.

With the experimental technique here employed and in view of the fundamental similarity between the clinical features of all the animals treated, the therapeutic effect must essentially be ascribable to vitamin B₆. It may be, however, that in the combined treatment the other vitamins may have a supplementary favorable effect on a couple of the symptoms.

The prevention of the degenerative changes in the central nervous system is ascribable to vitamin B₆ alone. In gastrectomized pups, presumably the preventive dose required for this effect will be between 60 and 90 γ per kg per day (cf. p. 93, Fouts and collaborators). When, in spite of the protracted treatment, one animal (in Group II) still has presented slight microscopic changes in the central nervous system, it might be due to various factors. In this connection, however, it is only reasonable to attach more importance to the fully positive result of the treatment as far as the central nervous system is concerned in the other animal within the same group. In particular the long duration of the experiment has to be taken into consideration as compared to the far more severe character and the more rapid, spontaneously fatal, course of untreated gastroprival pellagra.

Vitamin B₆ has played a predominant role also in counteracting the other symptoms amenable to treatment (skin and hair changes, fall in the red blood count). But neither treatment with this vitamin alone, nor its combination with the other vitamins B has been able completely to prevent the appearance of these symptoms. So, as far as these symptoms are concerned, either the dose of vitamin B₆ here employed has been too small (*i. e.*, it then ought to be over 140—175 γ per kg per day), or the gastroprival pellagrous symptom complex has to be looked upon etiologically as a plurality. The presence of the latter possibility is evident from the fact that in both experimental groups some of the symptoms remained therapeutically refractory, namely: inhibition of growth, emaciation and microcytosis.

Our therapeutic experiments have made it practicable from the *specific neurocutaneous symptom complex (experimental gastroprival pellagra) to separate a group of symptoms representing entirely or in part the clinical state of vitamin B₆ deficiency.*

According to Fouts, Helmer & Lepkovsky (& Jukes), a vitamin B₆-free diet produces in dogs a morbid condition that is charac-

terized especially by microcytic hypochromic anemia and changes in the nervous system (general convulsions, muscular spasms, epileptic attacks), besides inhibition of growth, fatty degeneration of the liver and hyperplasia of the spleen and bone marrow; but microscopic examination of the central nervous system appears not to have been made. [In rats this diet induces, among others, characteristic symmetrical loss of hair and dermatitis, localized especially to the paws, nose and ears (acrodynia) and epileptiform attacks (Eddy & Dalldorf).]

Within the gastropival symptom complex, as mentioned before, vitamin B₆ counteracts only — and a little inconstantly — the fall in the red blood count, but neither the decrease in the hemoglobin percentage nor the microcytosis. Further, the gastropival changes in the central nervous system — the appearance of which was prevented entirely by vitamin B₆ in 3 out of 4 cases — are clinically and morphologically of severe degenerative character whereas epileptic attacks have never been observed.

So even in the same species — the dog — there is a clinical contrast between two groups of symptoms of exogenous and endogenous nature respectively, both amenable to treatment with vitamin B₆.

Our previous therapeutic experiments on gastrectomized pigs showed that vitamin B₁ as well as lactoflavin and nicotinic acid are ineffective against the gastropival neurocutaneous symptom complex. This observation forms a contrast to the experiences from the clinic and from the experimental conditions of exogenous vitamin B deficiency. In our experiments, on the other hand, nicotinic acid had a pronounced effect on the pellagrous state of the animals when the part of the stomach (cardia + fundus) was not included in the resection. The therapeutic experiments here reported have established that *vitamin B₆ exerts its therapeutic effect independently of the stomach, i. e., independently of the specific antipellagrous factor attributed by us to the stomach.* Thus vitamin B₆ differs from other vitamins B in being able *directly* to counteract the appearance of the degenerative pellagrous changes in the central nervous system. Accordingly, vitamin B₆ may be designated as the specific antimyelopathin.

According to our latest investigations published (1942, 1943), elective total resection of the fundus produces in pigs the same

severe pellagrous changes in the central nervous system as does total gastrectomy. So the fundus is primarily decisive of the appearance of these changes. On correlation of this observation with the effect of vitamin B₆ on the changes in the central nervous system in totally gastrectomized pups, it seems reasonable to assign *an important role to the fundus as to the behavior of vitamin B₆ in the organism*. Then, whether vitamin B₆ is liberated or formed from the food through the function of the fundus, or whether the absorption of this vitamin directly or indirectly is dependent upon the presence of this part of the stomach will have to be left an open question.

Summary.

The symptoms of experimental gastroprival pellagra (= the neurocutaneous symptom complex) are influenced strikingly by parenteral preventive treatment with vitamin B₆, the clinical features being constantly mitigated and qualitatively altered by this therapy. Thus, administration of vitamin B₆ was able in 3 out of 4 experimental animals to prevent — and in the fourth animal strongly to inhibit — the appearance of the degenerative changes in the central nervous system, reduce considerably the skin and hair changes and also, in most of the cases, the fall in the red blood count. On the other hand, the vitamin B₆ therapy had no effect on the inhibition of growth, emaciation, fall in the hemoglobin percentage and microcytosis.

Administration of the other vitamins B together with B₆ has, at the most, a slight supplementary therapeutic effect on a couple of the symptoms.

Accordingly, vitamin B₆ has to be characterized as the only one of the vitamins B investigated so far by us (vitamin B₁, nicotinic acid and lactoflavin) which by itself alone exerts a distinct therapeutic effect on experimental gastroprival pellagra, i. e., exerts its effect independently of the stomach.

Vitamin B₆ is taken to be the specific substance counteracting directly the appearance of endogenous myelopathy («specific anti-myelopathin»).

On comparison of the results of our previous experimental

studies with the present, it is evident that the fundus must be of significance to the behavior of vitamin B₆ in the organism.

The present experiments indicate that the gastroprival neuro-cutaneous symptom complex constitutes an etiological plurality in which vitamin B₆ deficiency is the most essential cause.

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On Intraventricular Conduction Disturbances in the Specific Conduction System of the Heart.

By

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Introduction.

Our knowledge of the normal and pathological function of the specific conduction system of the heart is almost entirely due to electrocardiographic studies. And, as a matter of fact, in by far the greater number of cases disturbances in the function of the conduction system only manifest themselves with certainty in electrocardiography.

The disorders which come into question are reduced or abolished conduction in parts of the system as well as a stimulus conduction along paths where the conduction usually takes place in another way as regards time and direction. The latter phenomenon will often be a result of the former.

Normally the conduction system has not the same power of conduction at all times, its function in itself causing a variation. This is revealed in the absolute and the relative refractory period corresponding to an entirely abolished and a delayed conduction respectively. At normal physiological frequencies it is of no significance for the aspect of the auricular and ventricular complexes, that is to say, no change occurs in these with rising or falling frequencies. As far as the terminal complexes are concerned there may, however, be found small changes at increased action of the heart.

In all instances this is partly due to conduction phenomena. The aspect of the initial complex is in the first place determined by the function of the conduction system and in less degree by that of the heart muscles proper, while the terminal complexes are also affected by changes in the latter (25).

The elucidation of the nature of the conduction disturbances has especially taken place through the study of the conduction conditions between the auricles and ventricles through the auriculoventricular fascicle, the latter being the only connection between these sections of the heart, which renders conditions relatively simple. The diagnosis and classification of the conduction disturbances in the auricles and ventricles, which is of great interest, meets with considerably greater difficulties for the following reasons: 1) The diffusion of the conduction system is much more scattered, ramification and anastomosing much more pronounced than in the narrow connection between the auricles and ventricles (15, 19). 2) The specific conduction system is not the only one possessing the power of conduction, for the ordinary heart muscles will also conduct impulses, though at a much slower rate. It is said to be as 1 to 10 compared with that of the specific conduction system. According to recent investigations the rate of conduction in the ordinary muscles of the heart is, however, perhaps somewhat higher than was first supposed (25).

As already indicated, conduction disturbances are due to entire or partial blocking of the paths of conduction. It will therefore be convenient as a starting point for the study of the intraventricular conduction disturbances to divide these into the forms of block known from the auriculoventricular bundle. These are: 1) A—V block of the first order where the conduction from auricles to ventricles is delayed, but in which all impulses are conducted. The conduction time may vary. 2) A—V block of the second order, where some of the impulses are not conducted to the ventricles, one or several beats dropping out. This may happen either in connection with a gradual lengthening of the conduction time up to entire blocking, or by constant conduction time for those impulses which are conducted, and results in ventricular contraction. In this way arise the two forms of blocking of the second order which are termed Type I (Wenckebach) and Type II (Mobitz). 3) A—V block of the third order, complete block in contrast with the two

preceding incomplete blocks. Here there is a complete separation between auricles and ventricles as far as the conduction of impulses is concerned.

This division is based on experimental investigations on animal hearts as also on clinical pathologico-anatomical investigations on human subjects and an excellent agreement has been found between the various findings. The same possibilities of explaining the mechanism of intraventricular block are of course also present, but for the above-mentioned reasons the observations are considerably more difficult to interpret. To this must be added the fact that the localisation of the intraventricular block is of great significance for the form of the electrocardiogram.

Previous investigations and reports.

At first the blocking of the main branch of the conduction system to each of the ventricles attracted attention, and the criteria for these were established. Later on views on the blocking of the ramifications were developed, where the block phenomena were assigned to the finest ramifications of the Purkinje fibres. These two types of intraventricular block are both characterised by a QRS which is wider than usual, namely at least 0.10 second. According to the «Nomenclature and Criteria for Diagnosis of Diseases of the Heart» (21) which K. Larsen & Skulason (24) also accept, one must even demand duration of 0.12 second or more. The bundle branch blocks show large deflections while in contrast herewith arborization blocks have very small deflections, the amplitude being below 0.4—0.5 mV.

The bundle branch blocks are actually in a number of cases due to blocking of one of the main branches caused by a small circumscribed process; but in other cases widespread changes in the ventricles must be taken into account, with predominant localisation on one side. This fact perhaps explains the rather considerable difference (23) there is in the prognosis for this form of blocking. Of recent years some reserve has been shown with regard to the localisation diagnosis of branch blocks and they are merely divided into types according to the form of the electrocardiogram (Wilson, Bayley 20).

As far as the arborization blocks are concerned the changes are always widespread.

While blocks with the above-mentioned localisation and extension give typical electrocardiographic changes it is otherwise with the other intraventricular blocks. For frequently such disturbances of conduction will not produce changes in the electrocardiograms so large that they exceed the normal range of variations. Notching, slurring, and nodes in the initial complex which might suggest intraventricular disturbances in conduction are not considered to be of any great diagnostic value (24). Of greater importance are perhaps complexes resembling those found in bundle branch block but which do not reach or exceed the duration required for the diagnosis of the two above-mentioned types of intraventricular block, transitions to these being seen (25). Occasionally it is possible in such instances to get QRS complexes in precordial leads with an amplitude of over 0.10—0.12 second, while this is not the case in the normal leads from the extremities (23). We then speak of a localised block, since it is supposed only to manifest itself in leads from areas which lie near the place in the ventricular wall where the block is. Besides in these instances there is under special circumstances a chance of diagnosing an intraventricular block, namely when the block changes. This occurs when one has electrocardiograms from various points of time with differences in the complexes, especially as regards the duration of the initial complexes. In one and the same electrocardiogram, block can be diagnosed when the blocking is constantly changing, that is to say, with a block of the second order, or perhaps with a block of the first order with varying time of conduction. On the other hand, a block of the first order with a constant delay in conduction, and a block of the third order cannot be safely diagnosed and, moreover, cannot be distinguished from each other, amongst other things on account of the power of conduction of the ordinary heart muscles, which only allow of small delays in the conduction of the stimulus. A block of the second order will show in the electrocardiogram by the indiscriminate occurrence of complexes of different aspects though they have arisen in the same manner, as can be established from the situation and form of the P waves.

Some of the first observations of such complexes were made by Lewis (1) who found them in the supraventricular extrasystoles, where otherwise as a rule the same ventricular complexes are met with as in the normal beats. He found similar deviations in cases

of tachycardia. In later works (2, 3) he described the phenomenon both on experimental and clinical cases and proposed the term »aberrant complexes». It was observed that the changes were greatest in the complexes which followed most closely upon the normal ones. They were explained on the assumption of a difference in the time of restitution in the two main branches of the ventricular conduction apparatus. Kahn (4), Carter (6), Wilson (7), and Lutembacher (19) have likewise studied these phenomena and have arrived at similar results.

In the course of time several such cases have been described, and investigators have become more inclined to see in them something that comes within the range of the normal, caused by a normal relatively refractory period. Pardee (26) has advanced the view that an auricular impulse arising in an abnormal place enters into the auriculoventricular node in another place than the normal one and also leaves it in an abnormal place. This is supposed to give rise to a deviation in ventricular conduction which causes an aberrant complex. This might explain the aberration in some cases of extrasystole and tachycardia of supraventricular origin. It must be added that the second beat after a pause has proved always to be less well conducted than the succeeding ones, even though the distance between these is not greater than between the first and the second beat (25). An extrasystole, as we know, will always represent such a »second beat».

The deviations from the normal complexes here in question are, however, all on the whole small, and there can hardly be any doubt that a number of cases with markedly aberrant complexes in supraventricular extrasystoles which do not occur very early after a normal beat are due to an abnormally long, relatively refractory period, that is to say, a block of the first order or perhaps a temporary complete block, a block of the second order (1, 19, 23).

Outside extrasystoles and tachycardia too, a number of aberrant ventricular complexes have been reported, both occurring spontaneously and produced experimentally, for instance by sinus or bulb pressure, atropinisation, and other medicamentous effects. Here the 2:1 branch block, which, however, does not in principle differ from other forms of intraventricular block, has especially attracted attention. It has been described in most detail by Winterberg, Wenckebach & Winterberg (14), Leinbach &

White, Kelly, Norman Boyer and Geill (22). For the sake of completeness it need only be mentioned that the increase in duration which may occur in the initial complexes with deviation of the electrical axis of the heart, may perhaps be due to a change in the absolute and relative length of the conducting paths owing to hypertrophy, while there need not be a diminished rate of conduction (25). The types of electrocardiogram described by Wolff, Parkinson, and White, having an A—V conduction time less than 0.12 second in conjunction with a wide and deformed initial complex, are explained in a special way into which we shall not here enter in more detail, and are not real intraventricular disturbances of conduction.

In a number of papers dating from 1923 to 1927 (10, 11, 12, 13) Stenstrom has thoroughly studied the intraventricular block. As a result of his partly clinical, partly experimental investigations he found that there are forms of intraventricular block similar to those known from the auriculoventricular bundle, but that intraventricular blocks, despite the fact that they must be supposed not to be very rare, are not often diagnosed (apart from bundle branch and arborization block). This also applies to the incomplete blocks which would otherwise render a diagnosis possible.

He describes two cases which resemble complete blocks but prove to be incomplete blocks of the first order, the electrocardiogram showing normal complexes at reduced frequency. This is explained by an abnormally long period of restitution in one of the branches of the conduction bundle, exactly as in Lewis's interpretation of extrasystolic aberrant complexes. Further, two cases are reported of the occurrence of abnormal ventricular complexes with normal rhythm and otherwise normal electrocardiograms.

Own Observations.

Below we shall describe some instances of electrocardiograms in which the assumption of various degrees of intraventricular block will explain the special peculiarities. The changes are of rather rare occurrence, having been found in several hundred cardiograms of old people (over 60 years of age) in whom abnormal electrocardiograms are otherwise frequent. This agrees with previous reports.

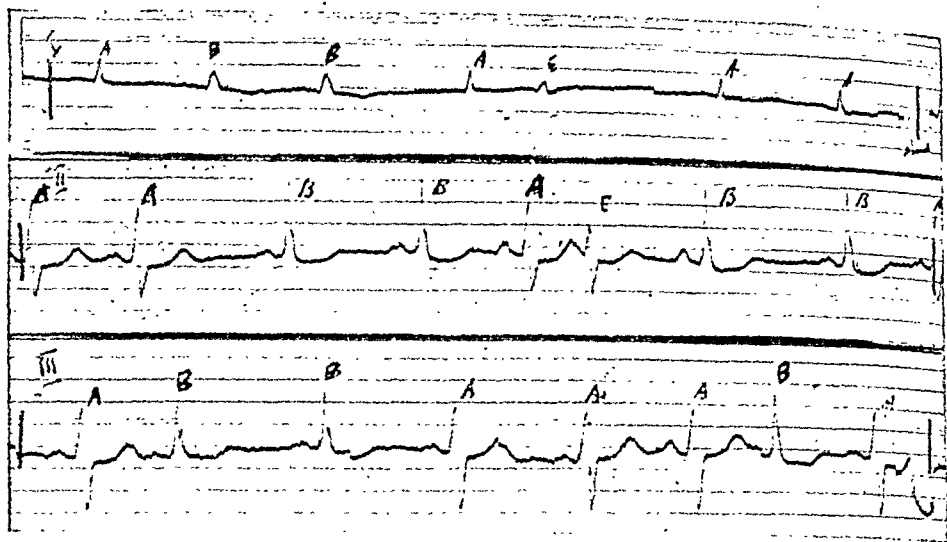


Fig. 1. Case I. The electrocardiogram shows in the three leads various complexes due to an intraventricular block of the second order. The complexes are denoted A, B and E.

Case I (Fig. 1).

2 (3) kinds of ventricular complexes occur in every lead. The action is very irregular and it is not possible to distinguish with certainty the one form of the extrasystoles and the other form of the ordinary complex. In all leads P—Q is about 0.15—0.16 second. The P waves in II and III are somewhat variable, especially as regards height but, it would seem, bear no definite relation to the two forms of ventricular complexes. The size of P—Q shows that all the ventricular complexes are elicited from the auricles.

If we term the two kinds of ventricular complexes A and B, the QRS of A I is found to be 0.08 second, that of B I 0.09 second. It is not possible, however, to decide with certainty the correlation between the A and B complexes in I and the A and B complexes of the other leads. In the denotation account has been taken of the aspect of the terminal complexes.

The QRS of A II is diphasic 0.11 second, T being large and positive, while the QRS of B II is 0.11 second monophasic with notching of the descending part, S—T below the isoelectric line, T diphasic. Finally the complex E II is a somewhat modified A II as a result of its coming in the relatively refractory period after the previous A. E I, on the other hand, is more probably a ventricular extrasystole. In A III the QRS is diphasic 0.11 second. B III is monophasic, QRS 0.11 second. As to S—T and T, similar conditions apply to both complexes as to A II and B II.

The electrocardiogram is derived from a man aged 77 who died of cancer ventriculi. He had symptoms of a peripheral arteriosclerosis, blood pressure 200/85, but no other clinical symptoms of circulatory affections. No treatment with digitalis. Section showed: Hypertrophy of the left ventricle, severe sclerosis of the coronary arteries which were in part im-

passible. The myocardium in places somewhat pale without actual infarction. In addition, Cancer ventriculi and pneumonia.

The explanation of this peculiar electrocardiogram is probably that a larger branch of the conduction bundle is blocked, causing the B complexes to appear. The duration of the complexes is just on the borderline of the highest allowable, but as there is at the same time, in the complexes which must be regarded as «normal», namely A, a left-sided preponderance, the wide QRS cannot quite simply be taken as a sign of blocking. The B complexes, however, resemble those seen in concordant forms of branch block. The block cannot occur as a result of a normal refractory period, since the time interval by far exceeds this. Furthermore, the abnormal complexes (B) do not always appear after shorter intervals than the normal complexes (A). Only E II must, as previously stated, be supposed to have appeared in this way.

Hence we have here an intraventricular block of the second order.

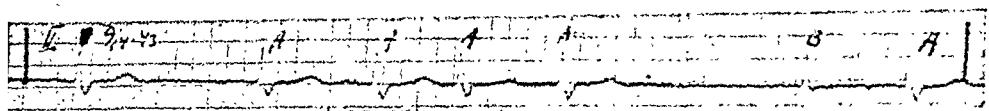


Fig. 2. Case II. The electrocardiogram shows in lead II two types of complexes seemingly depending of the interval between the cycles. The complexes are denoted A and B.

Case II.

Electrocardiograms from this patient taken in the course of 3—4 months showed a very irregular action which was partly due to an aphasic sinus arrhythmia, now and then probably with sinuauricular block, partly to the occurrence of supraventricular extrasystoles. The absence of constant P—P intervals often renders it difficult to ascertain of what kind the complex is in each single case. This is, however, of less importance for the explanation of the phenomenon that the initial complexes vary a good deal, so that the variations bear a certain relation to the length of the pause which precedes the beat.

As a rough characterisation of the initial complexes it may be said that in lead I they are monophasic upright, plump, with slight notching. In leads II and III there are R waves of fairly normal appearance and duration, while the S waves here are plump and wide in most complexes. The T waves are negative in III.

Fig. 2 shows lead II of the electrocardiogram of the 9/4—43. P—Q is about 0.13—0.14 second (the P waves are more distinct in the other leads). QRS of the A complexes is 0.11 second of which S constitutes 0.08 second. S is 0.3—0.4 mV. The distance between the A complexes fluctuates between 0.48 and 0.66 second, corresponding to frequencies between 120 and 100, which does not usually give rise to aberrant complexes when the conduction conditions are normal. In the B complex QRS is 0.08 second, and S is only just suggested. R has the same amplitude as in the A complex. The distance between the A immediately preceding B, and the B

complex, is about 1.50 second, which corresponds to a frequency of about 40. No P wave can be seen before B, so this initial complex might be regarded as a «nodal escape», where a minor aberration is not rarely seen as a result of the abnormal place of origin of the stimulus. The P waves, however, are not seen, either, before any of the other complexes in this lead, while they are distinct enough in other electrocardiograms which show the same conditions as those just mentioned, e. g. in fig. 3. The electrocardiogram dates from the 14/5 43. Here too we have lead II. Here the A complexes have : duration of 0.11—0.12 second against the 0.09—0.10 second of the B complexes. The corresponding S waves are 0.4 mV and 0.2—0.3 mV. The distance between B and A corresponds to a frequency of 60—70, while between the B complexes the distance answers to a frequency of 40—50. Quite similar conditions are found in the other electrocardiograms taken. From considerations of space only lead II is shown. Intravenous injection of 2 mg atropine sulphate caused a tachycardia of about 100—110 with complexes of the A type: sinus pressure elicited a small attack of convulsion so that the electrocardiogram was a failure.

The electrocardiograms are derived from a woman aged 72, who had a complete right-sided hemiparesis with aphasia. Blood pressure 120/70—95/50. Stethoscopy of the lungs showed nothing abnormal. In the clinical examination the heart was not enlarged. No sign of circulatory insufficiency.

The variations in the initial complex may be interpreted as the result of a relatively refractory period which far exceeds the normal. The connection with the pause preceding the beat favours the assumption that there is not a complete interruption but an incomplete block of the first order. Similarly A—V block of the first order shows a variation in the time of conduction depending on the frequency. The form of the complexes in leads I and II would seem to show that we are here confronted with a transition form to a bundle branch block of «the ordinary type», if the electrocardiogram can be associated with bundle branch block at all.

Case III.

The characteristic feature of this electrocardiogram (fig. 4) is the occurrence of two different forms of ventricular complexes which alternate quite regularly. The P waves are of the same shape and P—Q of the same size,



Fig. 3. Case II. Lead II from an electrocardiogram 5 weeks later shows still more distinctly two types of complexes depending of the interval between the cycles. Thus we have an incomplete block of the first order.

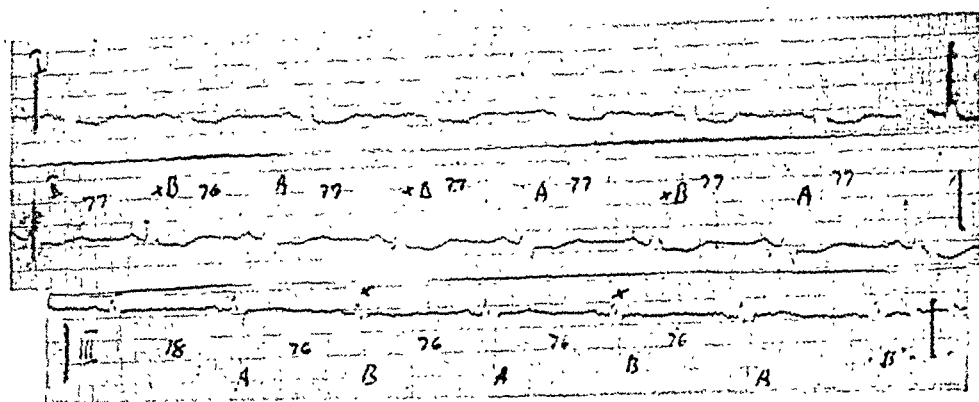


Fig. 4. Case III. In all three leads the complexes A and B alternate regularly due to intraventricular conduction disturbances. Thus we have a 2:1 block in a part of the intraventricular conduction system.

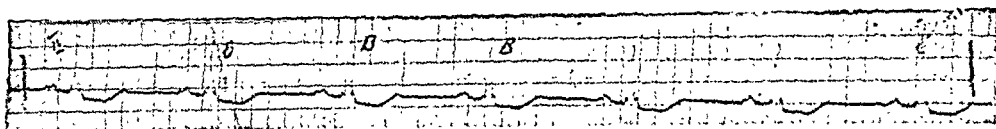


Fig. 5. Case III. Here the complexes are identical owing to complete blocking after treatment with digitalis.

about 0.16 second. The distance between the complexes is such that there must be a sinus rhythm. The A complexes have a somewhat plump S II, while there is no S in the B complexes in this lead. In lead III the A complexes have an S of 1.2 mV, while the B complexes are about 0.9 mV. The R wave is a bit wider and somewhat more notched in the B complexes than in the A complexes. The duration of QRS is around 0.09 second. S—T is depressed and bent downward in leads I and II where T is diphasic. This is most pronounced in the B complexes. T III is low but positive in A, in B it is isoelectric.

An electrocardiogram taken two days later shows the same forms of ventricular complexes, with only a few of the B type. The electrocardiogram shows left-sided preponderance.

Tabl. fol. dig. 1 × 3 was now administered to the patient for some days, after which only B complexes appeared. At the same time the depression of S—T became somewhat more pronounced (fig. 5). Sinus pressure and injection of 2 mg atropine sulphate altered nothing in these conditions.

The electrocardiograms are from a woman aged 78 who, like the preceding patient, had a right-sided complete paresis and aphasia. The blood pressure was 230/120. The limits of the heart could not be clinically determined. There was no sign of cardiac insufficiency.

This is a case of electric alternance due to an intraventricular conduction disturbance, which became stationary during treatment with digitalis,

even after a cumulative effect of the digitalis must be supposed to have ceased. Hence we are here faced with a 2:1 block in a part of the intraventricular conduction system. An electrocardiogram (not shown) exhibits a minor degree of blocking and after treatment with digitalis the block becomes complete (block of the third order). Though, as previously stated, it may also be regarded as a block of the first order. The difference in S—T in the two kinds of complexes may also be connected with conduction disturbances.

Discussion.

From anatomical and pathologico-anatomical points of view it might be expected that blocking of the paths of conduction in the ventricles must be frequent phenomena, even though the conduction system perhaps is better protected against harmful influences than the heart muscles proper, amongst other things by the greater possibilities of blood supply (17). As previously mentioned, apart from bundle branch and arborization block, intraventricular block is rarely diagnosed, and this is due to the fact that the areas which are affected by conduction disturbances often have a localisation and extension which do not give rise to sure pathological changes in the electrocardiogram. The incomplete intraventricular blocks, too, are rarely noticed but this is not explained, either, by simply assuming that they seldom occur, if the relative frequency with which they appear among the A—V blocks is taken into account. Perhaps a number of them escape attention when they occur in extrasystoles, the complexes being erroneously taken to be of ventricular origin on account of their aberration. This is especially the case when the P waves are indistinct. Conversely it must of course be ascertained whether the P—Q intervals have a length warranting the conclusion that the P wave and the ventricular complex are of one set, that is to say, that P—Q exceeds 0.12 second and does not vary. Finally the diagnosis of intraventricular block may be impossible because blocks with a suitable different localisation may cancel each other's effect on the electrocardiogram (Winterberg & Rothberger cit. 25).

A consideration of the anatomical conditions in conjunction with the view according to which the conduction in the specific muscle system is auxomerous (5) will explain the above-mentioned phenomena. That the conduction is auxomerous means that the

conduction of the stimulus to an area takes place normally as long as there is merely one fibre to this which is intact. Hence no changes take place in the electrocardiogram under these circumstances, either.

By means of a couple of diagrams in which the conduction system with anastomoses is sketched it will easily be seen that blocking of a single fibre, for instance at the point P, even if it constantly changes, will not give rise to any changes in the electrocardiogram owing to the numerous almost equal possibilities of conduction when P is among the finer ramifications (fig. 6). Further, it will be seen from this figure in which the hatched area denotes complete blocking but of a size that does not give rise to sure pathological changes, that incomplete blocks of the fibres in the vicinity of this area need not, either, give rise to changes in the electrocardiogram, since the stimulus is not compelled to go by widely devious ways in order to get to point A. In fig. 7 both the hatched areas are supposed to be completely blocked so that only a single path L can pass between them. The size of the areas thus presupposes that each of them separately does not give rise to sure pathological changes in the electrocardiogram as long as L is intact. If, however, L is now blocked the stimulus will be considerably delayed on its way to A, which will elicit a ventricular complex of another appearance than those usually occurring, that is to say, an aberrant complex.

Such combinations, however, are not common, for anatomical reasons, as is easy to see. The same experience has been gained from animal experiments where it has been attempted to produce incomplete blocks. Thus for instance Stenström, in the above-mentioned works only succeeded in evoking the desired changes in a very few of his experiments, in spite of an excellent technique. This view is supported by pathologico-anatomical observations (17—25) showing that many scattered processes may be found in the myocardium without any essential changes appearing in the electrocardiogram, as also by the fact that in cases where block has occurred just before death only old scars have been found. The fresh process causing the final block only need have a very small extension so that there is nothing strange in the occurrence of a block from day to day without at any rate macroscopically fresh traces (25). A microscopical demonstration meets with very

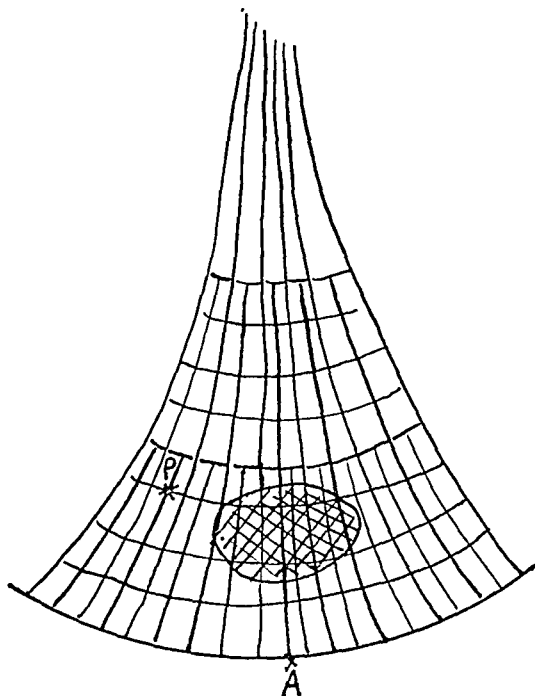


Fig. 6.

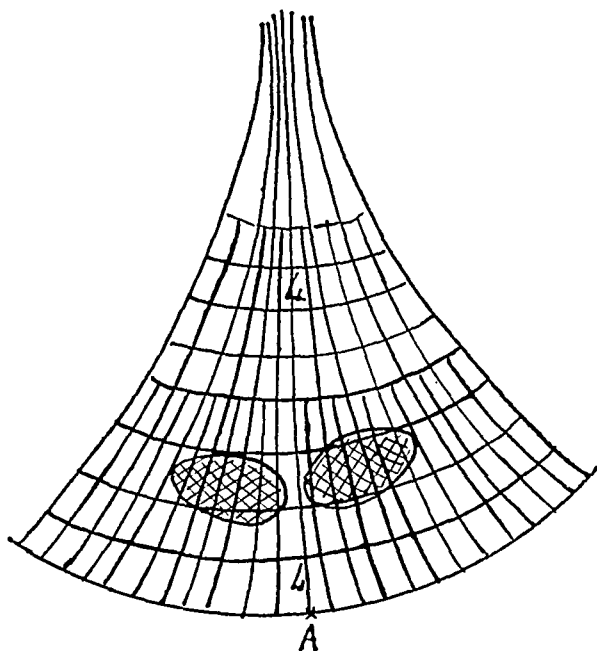


Fig. 7.

The figures show schematically the distribution of the conduction system and its anastomoses. The hatched areas denote complete blocking. Further explanation in text.

great difficulties when there is only a very defective approximate localisation to go by.

The significance of the demonstration of an intraventricular block is not in the existence of the block in itself, but in the fact that in such cases it is known that there is an organic affection of the heart, if intoxications with digitalis and quinidine can be excluded. In these instances the blocks are stated to be benign (23).

In the cases reported no effect of the vagus nerve on the phenomena has been demonstrated. In many of the previous investigations the view has been advanced that the vagus function had a bearing on the occurrence of aberrant complexes (6, 7, 13), so that both increased and reduced vagus tonus should be able to elicit these abnormalities. Several investigators (7, 8) state, however, that changes in the vagus tonus can only lead to abnormal complexes when there is already a latent conduction disturbance. Others have not been able to influence the phenomena by means of the vagus (22).

Some recent investigations go against the supposition that the vagus effect on the heart should extend any farther than to the auriculoventricular node, as vagus fibres have not been found further than up to that point, just as unambiguous proofs that vagotonica and vagodepressive substances affect the function of the ventricles are lacking (9, 15). In animal experiments electrical stimulation of the vagus nerve does not give rise to changes in the initial complex (18). Falling away or partial falling away of the vagus tonus, on the other hand, by causing a more rapid action of the heart may produce aberrant complexes under certain circumstances, without it being necessary to assume a direct effect on the conduction system of the ventricles.

Several investigators, amongst others Stenström, have stated that digitalis might evoke intraventricular block. In one of the cases reported here treatment with digitalis caused an increase of the block which persisted for several months after the administration of digitalis had been discontinued. This would seem to show that digitalis, even in therapeutic doses, may cause very deep-going changes, at any rate when the tissue is already pathologically changed (26).

The cause of the intraventricular conduction disturbances in the above-described cases is probably to be found in changes caused

by sclerotic processes in the vessels supplying the conduction apparatus. This agrees with what applies to branch and ramification block in the age classes in question and the findings at post mortem in the one case where such are available. In this connection it may be mentioned that some of the first animal experiments that led to the occurrence of aberrant complexes consisted in ligation of branches of the coronary arteries.

Summary.

A general view is given of intraventricular conduction disturbances with special reference to the mechanism in the occurrence of incomplete intraventricular blocks. Their relative rareness is pointed out and it is attempted to explain these peculiarities on the basis of the anatomical conditions of the conduction apparatus and the assumption of an auxomerous mode of conduction.

Electrocardiograms from 3 persons in the age-class 70—80 serve as the starting point. The changes in the electrocardiograms are interpreted as incomplete intraventricular block.

The cause of the pathological phenomena is assumed to be sclerotic changes in the arteries of the heart. The conditions essential to a possible vagus effect are briefly discussed.

Literature.

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The Artificial Kidney: a dialyser with a great area.

By

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(Submitted for publication October 6, 1943).

Purpose of the artificial kidney.

In the treatment of patients suffering from uremia resulting from renal insufficiency our first attempt will be, where we cannot remove the cause of this insufficiency, to restrict the formation of endproducts that have to be excreted by the kidney by giving a diet containing little albumen. Next we make all extrarenal factors influencing the secretion of urine as favourable as possible. We regulate the absorption of water, we control the composition of the blood and supply sodiumchloride as this is lost by vomiting. We give alkali in the case of acidosis, and so on. Furthermore we try to aid the circulation as much as possible by removing troublesome exsudates, etc.

If in spite of all these efforts the secretion of urine should remain insufficient, so that the endproducts of metabolism accumulate more and more in the organism, the urea percentage of the blood rises and the uremia gets more and more serious, we have come to the end of our resources.

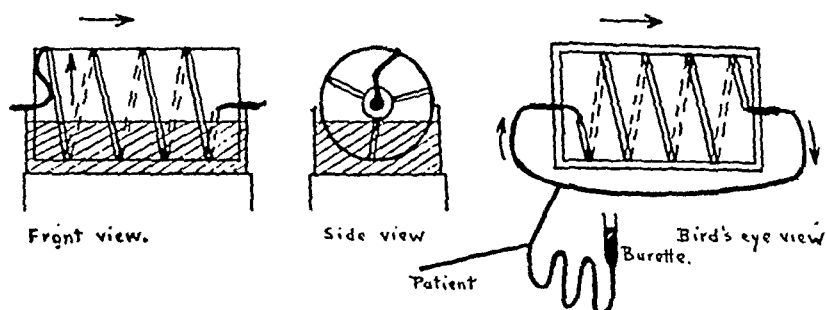


Fig. 1. A cellophane tube has been wound spirally round an aluminium cylinder. The blood within the cellophane always sinks to the lowest point. When the drum is rotating the blood moves from left to right.

Urea and other substances leave the body with the sweat, vomit and with the feces, and several ways of removing the toxic products responsible for the uremia without using the kidneys have been tried. However, all attempts at finding a satisfactory method have proved a failure.

All substances excreted by the normal kidneys can be dialysed, and as all these substances accumulate in the blood in uremia, one might try to remove these substances from the blood by dialysis. For this purpose the blood must be dialysed against a certain liquid through a system of tubes or membranes outside the body, and then brought back again into the patient's body. The

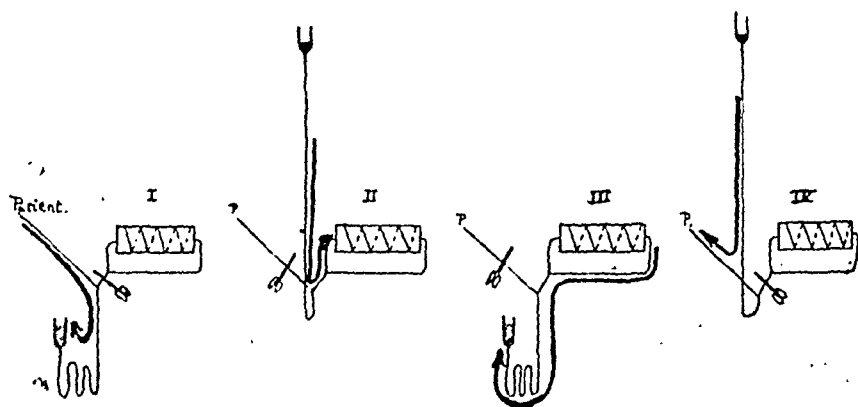


Fig. 2. I. Connection with dialyser shut off. Burette low: impure blood is flowing from the patient's body into the burette.

II. Tube to patient shut off. Burette high: impure blood is flowing from the burette into the dialyser.

III. Tube to patient shut off. Burette low: purified blood is flowing from the dialyser into the burette.

IV. Connection with dialyser shut off. Burette high: purified blood is flowing from the burette into the patient's body.

blood must be kept liquid by means of a substance preventing clotting. If all substances accumulating in uremia could be successfully removed, a person would be able to live without kidneys, in some cases so long till his own kidneys would resume their activity.

In 1912 and 1913 vividialysis was performed on animals by Abel, Rowntree and Turner 1) 2). The blood was pressed through a system of collodion tubes and subsequently brought back into the animal, clotting being prevented by hirudine.

Necheles (1924) 4) 5) and Haas (1928) 3) used other apparatus; the latter has applied vividialysis to human beings as well. The capacity was however much too small; in the course of a whole day of bloodwashing he could remove only 2 grams of urea.

In 1938 W. Thalhimer took up the problem again, armed with heparine and cellophane tubes. To our regret we do not know whether he has achieved a practical solution.

The apparatus.

Contrary to previous investigators we can prevent clotting by heparine, and we have excellent dialysing membranes; i. e. cello-

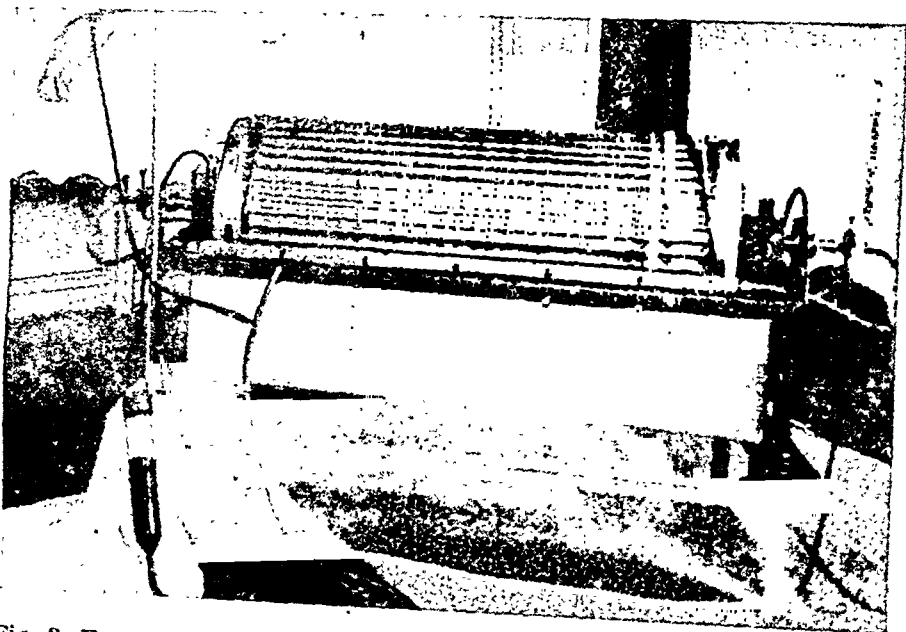


Fig. 3. Front view of the dialyser. The blood, contained in the 30 windings of cellophane tube which are plainly visible, covers the aluminium cylinder with a thin film. The cylinder has been provided with ridges.

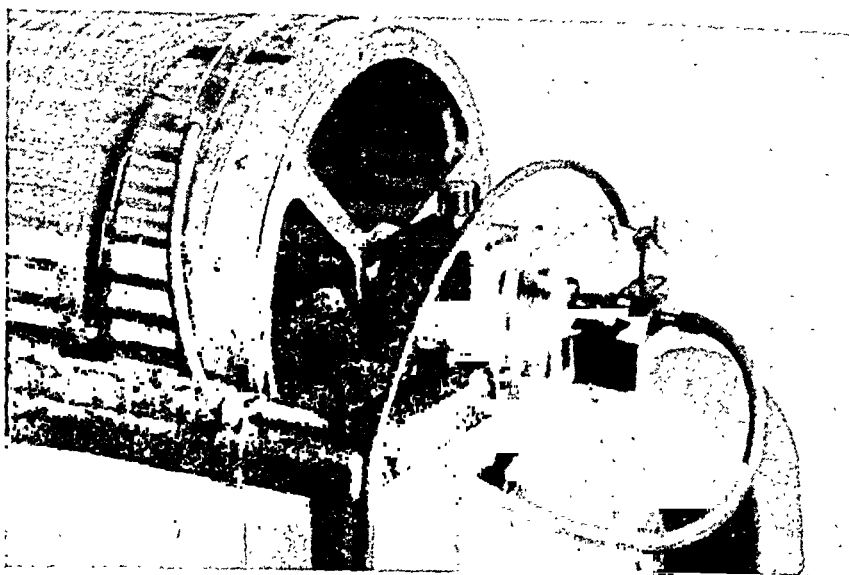


Fig. 4. Half lateral view. The cellophane tube passes into a rubber tube leaving the cylinder by the hollow axle, inside which the coupling is fixed after having been sterilised together with the tubes.

phane tube. The next step was the construction of a dialysing-apparatus with a small bloodvolume and a membrane large enough to rival the human kidney. We calculated that by using cellophane tube 25 mm wide we should need a length of at least 25 to 30 metres.

A principal difference between our apparatus and all previous ones results from the fact that we have not filled the tube system entirely, but that we cause a small quantity of blood to pass through a tube which is for the rest empty, so that the ratio area: volume gets much more favourable.

We pass over the various apparatus built in the last few years and shall describe only the artificial kidney in use at present.

The apparatus (fig. 3 and fig. 4) consists of a large horizontal cylinder, revolving with its undermost segment in a tank of rinsing liquid.

30 metres of cellophane tube have been wound spirally round the cylinder. The blood is in the cellophane tube, which has been for the rest evacuated and in which it sinks to the lowest point. With the cylinder rotating in the direction of the arrow in figure 1 the blood moves from left to right, continually seeking the lowest

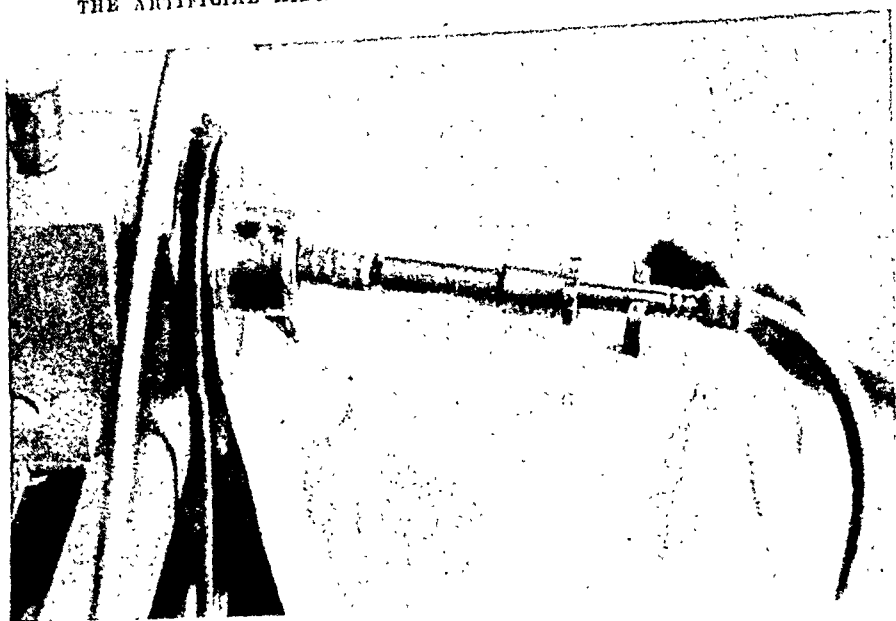


Fig. 5. A rotating coupling. The blood-tight joint is formed by a cotton packing being pressed on to the inner tube by means of a screw socket. Later on a counternut has been fixed on to the screw socket as well.

point. It leaves the cellophane tube by a rubber tube, carried through the hollow axle to the right (fig. 4), and it enters by flowing through the hollow axle on the left. In the hollow axles the rubber tube is interrupted by a rotating coupling (fig. 5).

Methods of dialysis:

1. *Continuously.* The blood may be let out of one bloodvessel, pass through the «kidney» and after dialysis be brought back into another vessel.

2. *Fractionnated.* The process is shown schematically in figure 2. A side tube has been attached to the circuit branching in two: one branch to the patient and the other to a burette. By raising or lowering the burette blood may be let into or out of the patient and into or out of the dialyser. In following this method only one single venapunction is necessary.

In the tube passing to the patient there is a cellophane window, through which may be seen directly whether there exists positive or negative pressure and by means of which one may check if blood is flowing.

Rinsing liquid. We use 70 to 100 litres of rinsing liquid warmed by a heating element to 37°—39° C, and clean though not sterile,

as cellophane is impermeable to bacteria. To avoid hemolysis the addition of glucose was necessary. At present we use the following composition:

NaCl	0.7 %
glucose	1.5 %
tapwater	97.8 %

Cleaning and sterilising. All parts coming in contact with blood, i. e. tubes, couplings and the cellophane are cleaned thoroughly and sterilised. They may be subsequently put together and kept filled with a solution of superol. Before use the superol is washed out with a-pyrogenic saline solution and all air is expelled from the tube.

Heparinising. At present we apply the following doses:
At the beginning: 400 mg of heparine in the «kidney»

400 mg » » » » patient (intravenously)

During dialysis: every half hour 100 mg.

Results of an experimental dialysis. When dialysing 500 cm³ of a 4.17 ‰ solution of urea 1.68 ‰ proves to be still present after 5 minutes; 2.49 ‰ or 1.24 grams have therefore been excreted by dialysis in these first 5 minutes. After continuing the dialysis for another 5 minutes 0.57 ‰ proves to be still present.

The dialysing area of our first kidney amounts to 17,000 square cms, and of our second one to 23,000 sq. cms, the total area of the human glomeruli being 20,000 sq. cms.

Patient.

Miss S., single, 29 years of age, consulted the ophthalmologist (Dr. Keiner) in December 1942 because her sight grew steadily worse. There was extensive edema of both papillae with hemorrhages and white foci in the retina. Dr. Dhont, specialist for internal diseases in Zwolle, found symptoms of a chronic nephritis with uremia: Tension 245/150. Urine: isosthenuria, hematuria, 2—3 ‰ of albumen. Urea percentage of the blood 110 mg %.

Under general treatment the urea percentage of the blood sank to 75 mg %, but on the 1st of March she had to be taken to hospital again. She vomited almost daily and her state grew worse, so that Dr. Dhont decided to give the artificial kidney a chance, well knowing there was nothing to lose, but perhaps a temporary improvement to gain.

On arrival at the Kampen hospital on the 16th of March 1943 her state was as follows:

By bleeding from the nose her Hb. had sunk to 35 % and was still going down rapidly. Pulse 100. Tension 220/140. Urea percentage 164 mg %. The breath smelt strongly of urine. The heart was enlarged towards the left, the ictus almost reached the axillary line, a gallop and a systolic murmur were audible. The patient sat up in bed and complained of palpitation and oppression of the chest. All over the lungs moist rhonchi were audible on the following days. All these symptoms have improved after we brought the Hb percentage to a normal level by transfusions of syrup of erythrocytes, during which each time we let an equal volume of the patient's blood escape for fear of causing pulmonary edema. During the subsequent course of the treatment the heart gave no more trouble.

As we did not know at all how our first patient would react to the dialysis we started with repeatedly dialysing small portions of blood. In the end we succeeded in keeping the percentage of urea at the same level for 26 days, after that no more serviceable veins were available.

Various complications have stood in the way of a clinical improvement. First of all the alarming bleedings from the nose, which necessitated repeated transfusions (totalling 14.5 litres), but which were subdued after reiterated cauterisations (Dr. Hinnen). On the 10th day a pericarditis became evident. Next, an angina, a painful parotitis and after that a very serious otitis media of both ears. The copious purulent secretion ran through the tubae Eustachii into the throat and excited the vomiting again which had just decreased a little. After treatment with sulfanilamides this too improved.

When a preparation of arteries (Dr. Kehrer) was necessary (all veins being ruined) very persistent hemorrhages arose from the subcutaneous connective tissue owing to the heparine.

After the 12th dialysis had become a failure, the artery being damaged, the urea percentage of the blood rapidly rose to 640 mg %, whereupon death followed.

At necropsy the following appeared:

Prof. Dr. J. J. Th. Vos was kind enough to examine the organs microscopically. The kidneys were very small, their weight was 80 and 67 grams respectively. The glomeruli showed all stages of degenerative changes up to perfect hyalinisation. The arterioles and capillaries showed serious sclerotic changes. The heart was very strongly enlarged with a mighty wall of the left ventricle. There was a purulent pericarditis without the signs of specific inflammation.

The lungs showed small cuneiform foci, reminding of old hemorrhagic infarcts. Prof. Vos however did not find necrosis in the centre, so that he is inclined to look on them as old hemorrhages. Clinically they gave no symptoms. Possibly they may be accounted for by hemorrhages (heparin!) around small emboli (clots), so that in future the blood will pass a filter before returning to the patient's vein.

In this patient we could not effect more than a slight prolongation of her life, but we have been able to collect valuable data.

Clinical symptoms of the uremia during treatment.

Miss S. did not make that deadly indolent and dull impression usually made by uremic patients. For the first four days after the greater dialysis she was often strikingly well and her mind was perfectly clear. The vomiting was temporarily less violent after the 5th to 9th dialysis.

Eyes: the ophthalmologist Rochat found on the 16th of March: strongly prominent papillae; veins widely dilated, many hemorrhages, and a small number of white foci radiating from the papillary region. The sight was very bad. During treatment the edema of the papillae disappeared nearly entirely and returned later. The hemorrhages were totally resorbed, and no fresh ones appeared. The white foci improved temporarily, increasing again later on. The sight improved so much that she could read the paper without any difficulty; this improvement remained until the last days of her life.

Bloodpressure. During the dialysis a sinking of the bloodpressure through shock could often be observed. Secondary effects of the heparine used probably accounted for this. Even when the shock was entirely overcome after dialysis by giving extra saline and blood, the bloodpressure remained at a lower level for the first few days after dialysis. On the graph (see figure 6) the dialyses are shown as columns: the white column indicates the quantity of blood dialysed, the shaded one the quantity of urea removed by dialysis. In the top of the graph the bloodpressure has been reproduced; here one sees that each time after a larger dialysis a lasting decrease of the bloodpressure occurs, e. g. from 180/110 to 145/100. After four or six days the bloodpressure returns to its former level. Possible causes are: 1. the removal of a tension-increasing substance from the blood by dialysis? 2. insufficiency of the left ventricle, or chronic shock. This was not in accordance with the clinical picture.

Urine. The hope that the patient's urine production would suffice to maintain a certain balance has not been fulfilled. One gets the impression that the urine production suddenly slackened off after the larger dialyses (see figure 6). This may be connected with the temporarily lowered blood pressure and the lowered percentage of urea in the blood after dialysis. Besides the amount of

urine passed per 24 hours decreased gradually during her stay in hospital, and the concentration of urea in the urine sank till it equalled the concentration of urea in the blood. This must be seen as a consequence of the progressive renal degeneration.

Never has there been a trace of hemoglobine in the urine after the dialyses. The sediment only seldom contained some leucocytes and erythrocytes.

Research on substances removed by the artificial kidney.

We are fully aware that urea is at the utmost only partly co-responsible for the clinical symptoms of uremia, but nevertheless we chose it as a measure for the results of the dialyses. Smaller molecules will dialyse more rapidly and bigger ones less so.

Urea: in the larger dialyses 24, 40, and 35 grams of urea were dialysed out respectively. The largest quantity of urea excreted by the patient per 24 hours with the urine amounted to 12 grāms.

Figure 6 shows how the percentage of urea after having risen rapidly in the beginning could be kept at the same level for 26 days, to rise again very rapidly after the treatment was discontinued.

In table 1 one finds the results concerning urea summarized once more. With fractionnated dialysis, where the blood circulates a few times through the kidney, the percentage of urea in the blood sinks to the concentration in the bath water. In continuous dialysis the blood flows only once through the kidney. It is there-

Table 1.

Percentages of urea in the blood and in the rinsing bath in mg per 100 cm³.

Dialysis N°.	I	II	IV	V	VI	VII	IX	X
Blood of patient before dialysis	172	217	303	319	288	339	324	324
Blood from kidney after dialysis	5	10	20	10	48	92	76	56
Percentage of urea in rinsing bath				10		30	58	51
Blood of patient after dialysis						278	233	333
	Fractionnated dialysis					Continuous dial.		

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Table 2.

Data concerning diverse retention products, Sulfamethylthiazol and Calcium in mg per 100 cm³ in dialysis N° X.

	Uric acid	Creatinine	Rest N	Urea	Sulfamethylthiazol	Ca
Blood of patient before dialysis	9.3 10	5.44	188	324	15.3	9.4 9.4
Blood of patient during dialysis	8.2	4.8		253		
Blood of patient after dialysis	7.4	4.7		233		
Blood from kidney during dialysis	4.7	2.2	51 50	45		
Blood from kidney after dialysis	4.1 4.8					6.7 6.5
Rinsing bath after treatment				51		

fore most often not being washed out so completely. Besides, the concentration of urea in the bath rises after prolonged dialyses, in future we shall change the water in the bath.

From this table one sees that uric acid, creatinine, rest nitrogen and urea are being removed from the blood by dialysis. The fact must be taken into account that the blood was flowing through the kidney only once before being infused again. In the rinsing bath, which was not changed, large amounts of these substances had accumulated towards the end of the dialysis (51 mg per 100 cm³ for urea).

Table 3.

Indoxylreaction after Jolles (most intense colour taken as 100).

Date Dialysis N°.	19/III II	21/III —	22/III III	23/III —	14/I X	4/V
Blood of patient.....		55		50		100
Blood from kidney	39		33			
Bathwater before treatment					0	
Bathwater after treatment					+ +	

Table 4.

Percentage of potassium in mg per 100 cm³ of the patient's blood serum (without a trace of hemolysis).

Date	16/III	23/III	12/IV	4/V
Potassium	18.8 and 19.6	18.8	17.8 and 18.1	85 and. Blood from 92 heart 8-hours after death.

The percentage of xanthoproteine in heparinised blood could not be read accurately.

From table 3 we may gather that indoxyl too is being removed from the blood by dialysis.

Percentage of potassium: we found it impossible to determine the percentage of potassium in heparinised blood plasma. One cannot but presume that the ionised potassium dialyses very rapidly, so that blood flowing through the kidney only once will probably lose its potassium entirely. This is important as in patients suffering from uremia an intoxication by potassium often seems to be the direct cause of death.

Dialysing into the blood of substances added to the bath water.

Through the dialysing membrane molecules go in and out. If it is e. g. desired to compensate the loss out of NaCl or glucose from the blood these substances are added to the rinsing bath.

Table 5.

Percentage of NaCl in the blood plasma in mg per 100 cm³ (calculated from the (Cl')).

Date Dialysis N°.	17/3 I	22/3 III	24/3 IV	26/3 V	28/3 IV	31/3 VII	6/4 VIII	8/4 IX	12/4	19/4 XI	4/5
Blood of patient	590 588	533 534	556 564	533 530	612 590	500 506	606 606		633 623	474 494	640
Blood of patient after dialysis ..				535 530		590 605		644 630			
Blood from kidney	658 652	642 649	633 632	575 585	560 562	639 638	748 750				
Bath water	650	650	650	570	570	700	700	700		700	

Table 6.

Percentage of glucose in mg per 100 cm³ of blood and bath water.

Dialysis N°.	IV	V	VII	X
Blood of patient before dialysis	95 95	98 107	114 114	104 106
Blood from kidney after dialysis	2	20 9	450 454	550 568
Bath water	0	0	1500	1500
Blood of patient after dialysis		80 85	301 301	189 200
	Bath water without glucose		Bath water with glucose	

From table 5 it is evident that Cl' has always entered the patient's body from the kidney, especially when the patient's percentage of salt was too low. In taking a retrospective view of the treatment we must admit that we did not always supply enough salt, although we gave it repeatedly. The loss of Cl' must be attributed to the vomiting.

In the dialyses N°s. IV and V no glucose had been added to the bath. The bloodglucose sank to very low values. In the dialyses N°s. VII and X (and other ones) glucose was being resorbed from the bath.

From the table printed above one sees that, given a well-chosen rinsing bath, a normal sodium percentage of the blood may remain

Table 7.

The sodium percentage of the blood serum in mg per 100 cm³.

Date Dialysis N°.	16/III	26/III V	31/III VII
Blood of patient before dialysis.....	329 330	312 309	305 292
Blood of patient after dialysis		328 312	
Blood from kidney ..			309 308

unchanged. If necessary, other substances which are as yet removed e. g. Ca, Mg, K, etc. may be added to the bath water.

The dialysable constituents of the blood will be regulated according to those of the fluid in the bath, a surplus being washed out, a deficit replenished.

Oxygen is resorbed very rapidly: already after a few windings one sees the blue blood get red.

Water.

It is not easy to form an opinion concerning the question what the water is doing. On both sides of the dialysing membrane osmotic forces are active. From the blood a mechanic force is acting as well. Towards the blood: the colloid-osmotic action of the blood-plasm. By adding glucose to the bath the detraction of water may probably be augmented.

Cultures of the blood

both from the patient as well as from the kidney after dialysis remained sterile.

Summary.

The artificial kidney is a dialysing-apparatus with a small blood volume and a dialysing area of about 20,000 sq. cms., in which the blood of a patient is cleared of retention products.

With one patient 24, 40, and 35 grams of urea could be dialysed out in 1.5, 4, and 6 hours respectively. Other retention products were removed by dialysis as well. This could be demonstrated for: rest N, urea, uric acid, creatinine and indoxyl.

We believe to be able to keep patients suffering from uremia and anuria alive so long as bloodvessels for puncture are available.

In the case of acute uremia the possibility exists for the kidneys to regenerate in the meantime. Sulfamethylthiazol and other substances with small molecules (poisons!) may be removed by dialysis as well.

Post Scriptum at the time of correction, January the 15th, 1944.

In table 5 the high value of 748 (check 750) mg % of NaCl is probably caused by infusion of saline a short time before the sample

for determination was taken. Further analysis of the rinsing fluid after dialysis no X gave the following results:

70 litres of bath water contained 42 mg of magnesium, 25 mg of phosphorus and 210 mg of potassium.

Two more patients have been treated with the artificial kidney, both were from a clinical point of view hopeless cases;

1. a man suffering from cachexia and uremia due to bilateral renal tuberculosis. 32 grams of urea were removed by one dialysis; on the next day he passed more urine than before the dialysis.

2. a man with an acute glomerulo-nephritis and oliguria, one of whose kidneys had been decapsulated without success. He passed into collapse and coma a few hours before the dialysis, from which he did not awake. 100 grams of urea were removed in 6 hours, the urea contents of the patient's blood showing a decline from 460 to 290 mg %.

In order to prevent thrombosis in the needles it proved advisable to start heparinisation 2 or 3 hours before the dialysis, by giving 200, 100 and 100 mg of heparine at hourly intervals.

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On the Price-Jones' curve in tape-worm anemia.

By

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(Submitted for publication November 19, 1943).

Introduction.

The distribution curve of red blood cells in pernicious anemia is, according to Price-Jones, quite different to that of healthy persons and in other kinds of anemia. Price-Jones considers the pernicious curve asymmetrical, often even grotesque, which has made him suspect the presence of a heterogeneous population of red blood cells in this disease. In a decomposition of the pernicious curve, according to Mogensen, a large main component can be distinguished, which consists of large blood cells, and in which the standard deviation is as a rule somewhat greater than the largest normal standard deviation, and as well one or two minor components. One of these, the left component is of constant appearance and thus consists of small blood cells, the other one, the so-called right component, is on the other hand, very rare and very small in size and composed of especially large cells. The great standard deviation, which is characteristic of the pernicious curve, is due in the first place to the appearance of the minor components. A curve similar to the one in pernicious anemia has been found by Mogensen in two cases of megalocytic anemia in idiopathic steatorrhoe but in no other states of anemia. Mogensen points

¹ The material for this investigation has been obtained from the Medical Clinic of Maria Hospital (Physician-in-Chief, Prof. Fr. Saltzman, M. D.) and from the Neuropathological Clinic of the University at Helsingfors (Physician-in-Chief, Prof. Ö. Holsti, M. D.). I am also indebted to Mr G. Elfving, Docent, D. Phil. for help in mathematical questions and to Mr T. Tötterman, B. Sc. Econ., who has assisted me with calculations.

out that it appears from accounts of the mean diameter of the red blood cells, which are to be found in Schauman's thesis on *Bothriocephalus anemia*, 1894, as if the distribution curve in *Bothriocephalus anemia* were asymmetrical in the same way as in cryptogenetic pernicious anemia. The Price-Jones' method has, however, never been tried in tape-worm anemia. I have used this method in 15 cases of the disease as such an investigation must undoubtedly be of a certain theoretical interest.

Methods.

Mogensen carries out the decomposition as follows. The inversion of the adjusted Price Jones' curve is transferred to the original adjusted curve in such a way that the two curves cover each other as much as possible. They cover each other almost totally when the distribution is normal. The distribution is symmetrical. The case is, however, different in pernicious anemia. The presence of the preponderate left component displaces the symmetry and on this account large or small parts of the two curves will be uncovered. Already the appearance of an asymmetrical distribution curve will give rise to the assumption that there are minor components. The approximate mean diameter, standard deviation and volume of the main component and the mean diameter and volume of the minor components can be graphically determined by the aid of Mogensen's method. The measuring of blood cells in my investigation has been carried out as in Hernberg's thesis on the size and shape of the red blood cells in people of various ages. 500 red blood cells have been measured in each case. To eliminate irregularities from the curve, caused by the measuring process, Mogensen uses a kind of gliding mean values. Hernberg, again, uses for the same purpose a redoubling of the class width. A further adjustment of curves with these gliding mean values has therefore not been considered necessary in my investigation. — The most important accounts of the 15 cases of tape-worm anemia are — to make it as short as possible — given in connection with the curves, the inverted curves being dotted. The number, sex and age of the patient are given in the top left-hand corner, and beneath the state of blood. The mean value of the poikilocytosis is calculated according to Hernberg. To the right of the curve the mean diameter (MD) of the red blood cells and the standard devia-

tion (s), calculated for the whole of the curve is given. Further, the volume (N_0), the mean diameter (MD_0) and the standard deviation (s_0) of the main component and the volume of the left component (N_1) in the 4 cases in which it has been possible to carry out a decomposition on account of the results of inversion are also given. The distribution curve of a healthy person (Mogensen's case No. 7) has been added for the sake of comparison and has here been entitled normal.

Results.

A survey of the blood values shows that the anemia is throughout hyperchromic and excepting in cases 6, 8, 12 highly developed. The mean diameter and the standard deviation of the red blood cells (MD and s) in the 15 cases investigated is very pronounced and surpasses the higher border of the normal, which is considered to lie at 7.65μ and 0.53μ . It may consequently be stated that the blood picture, in each case, is typically »pernicious».

Price-Jones and Mogensen have both found that in cryptogenetic pernicious anemia no correlation occurred on the one hand between the size of the standard deviation and the size of the mean diameter and on the other hand between the size of the mean diameter and the red cell count. But they found a noticeable conformity between the red cell count and the size of the standard deviation, as the standard deviation was largest in those cases which had the lowest red cell counts. The 15 cases of tape-worm anemia give exactly the same results when the corresponding values are compared.

If we investigate the curves, after having applied Mogensen's inverting method, we find that both curves in 11 cases (2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 14) almost cover each other. They may thus be considered fairly symmetrical without any decided minor components. The increased mean diameter and the displacement of the curves to the right, connected therewith, and the increased standard deviation of these curves suggest, however, that they do not belong to healthy persons. This is noticed, too, in a direct comparison with the normal curve. In 4 cases (1, 7, 13, 15) the asymmetry is, on the contrary, so great that the presence of a left component may be suspected. A minor component to the right, such as Mogensen believes himself to have noticed in some cases of

cryptogenetic pernicious anemia has not been traced in any of the 15 cases of tape-worm anemia.

The mean diameter of the main component (MD_0) is, according to Mogensen, the absciss of the symmetric axis of the original curve and the inverted curve. It is characteristic of the mean diameter of the main component in the 4 cases mentioned above that it is noticeably larger than the mean diameter of the whole distribution curve in the same manner as in Mogensen's cases of cryptogenetic pernicious anemia. This is in itself a proof of the presence of a left component, composed of small blood cells. The standard deviation of the main component is greater than the greatest normal standard deviation and yet not exceptionally great in Mogensen's cases of cryptogenetic pernicious anemia and the same is the case in these 4 cases of tape-worm anemia. In 3 of the cases in question (7, 13, 15) the left component is more noticeable as at least $\frac{1}{5}$ of the total number of cells belong to it. In the remaining case (1) the left component contains 72 cells, a figure which compared with the corresponding figures in Mogensen's cases of cryptogenetic pernicious anemia must be considered rather considerable.

As a control of the results obtained by Mogensen's inverting method should be important I have obtained directions from Dr G. Elfving of some analytical methods of which a short account will be given below.

In the first place cases have been chosen in which the asymmetry of the distribution is large enough to justify a trial of decomposition. For the purpose of finding an objective measure for the asymmetry we have calculated a so called skewness measure for the distribution. Several of the kind have been suggested in literature. Charlier's skewness measure

$$S_{Ch} = - \frac{\mu_3}{2s_3}$$

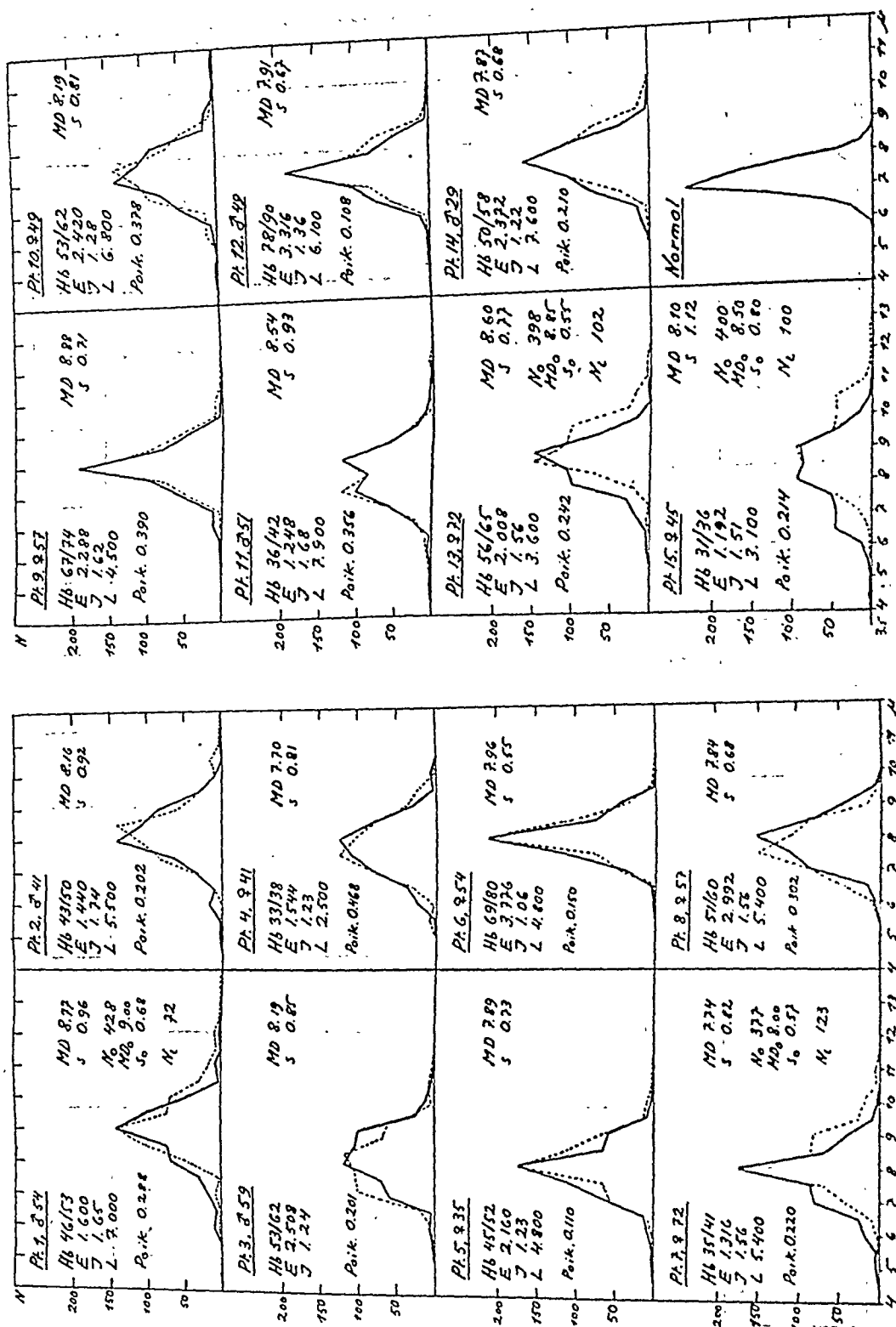
is well known. In it s is the standard deviation of the distribution and μ_3 its third moment of the mean value. Lindeberg's skewness measure

$$S_L = P - 50$$

is less known but preferable in many ways. P is the percentage in individuals for which x (here diameter of blood cells) exceeds its mean value. In a Gaussian distribution both these skewness measures have the mathematical expectation 0, while their standard deviations are given by the formulas ¹

$$\sigma(S_{Ch}) = \sqrt{\frac{1.5}{N}}, \quad \sigma(S_L) = \frac{30}{\sqrt{N}}$$

¹ J. W. Lindeberg: Über die Begriffe Schiefheit und Exzess in der mathematischen Statistik. Skand. Akt. tidskr. 1925, p. 106—127.



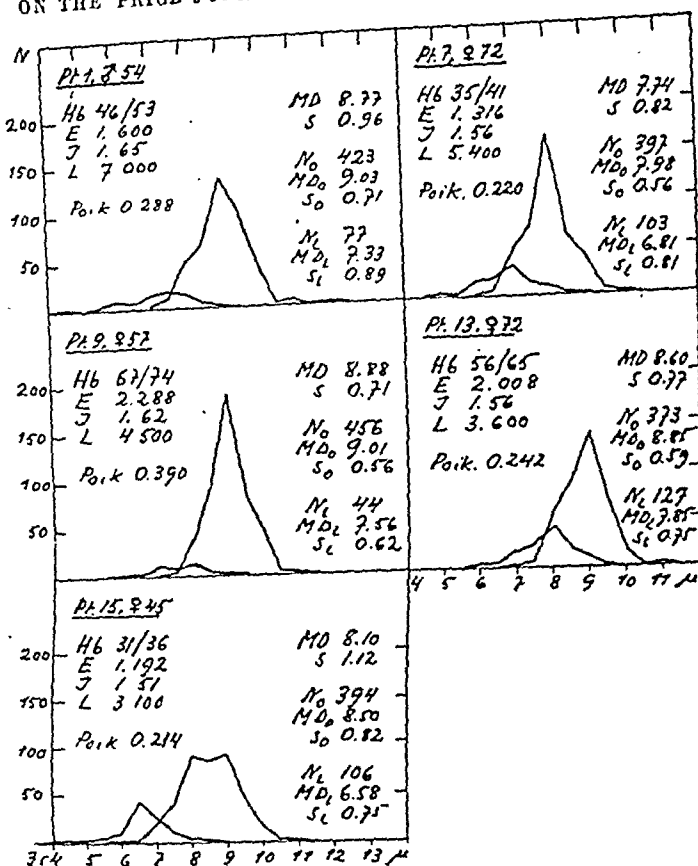
Decomposition according to Mogensen.

TABELL 1.

Pt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	±
Sch	.36	.28	.10	.04	.08	.03	.27	.01	.30	.02	-.02	.02	.26	.14	.26	.054
SL	8.1	1.4	1.2	2.0	1.2	-.5	7.9	1.1	4.8	-1.1	.9	1.5	3.8	3.4	4.0	1.34

TABELL 2.

μ	Pt. 1			Pt. 7			Pt. 9			Pt. 13			Pt. 15		
	N	N ₀	N ₁	N	N ₀	N ₁	N	N ₀	N ₁	N	N ₀	N ₁	N	N ₀	N ₁
4	1		1	5									1		1
4,5	—		—	4									3		3
5	1		1	17									2		2
5,5	9		9	4									5		5
6	7		7	19									12		11
6,5	7		7	25									46		3
7	17		15	78									17		19
7,5	30		12	83									24		41
8	66		50	173									51		10
8,5	73		16	66									93		89
9	140		67	40									86		85
9,5	100		136	8									12		91
10	45		100	1									148		45
10,5	3		45	8									70		16
11	7		3	1									17		3
11,5	—		—	—									—		—
12	1		1	—									2		1
MD, MD ₀ , MD ₁	8,77	9,03	7,33	7,74	7,98	6,81	8,88	9,01	7,56	8,60	8,85	7,85	8,10	8,50	6,58
s, s ₀ , s ₁	0,96	0,71	0,89	0,82	0,56	0,81	0,71	0,56	0,62	0,77	0,59	0,75	1,12	0,82	0,75



Decomposition according to Elfving.

The skewnesses, according to Charlier and Lindeberg, are given in tab. 1, also their mean errors (last column). If a divergence of about 3σ or more is considered significant the results of both the skewness measures will agree excepting in case 2. With regard to the shape of the curve and to the special precedence given to Lindeberg's measure it seems more correct to leave this case out of the group of skew distributions.

It now lies close at hand to imagine the asymmetrical distributions as composed of a main and a minor component, both perhaps normally¹ distributed. On the basis of this hypothesis a decomposition of the distribution of blood cells has been attempted in cases 1, 7, 9, 13, 15. An approximation system has been used for this purpose. It will be given in short below. The right side of the distribution may be considered almost free from the influence of the minor component. In consequence of the assumption and by the aid of so called probability papers approximate values for the number of individuals, mean value and standard deviation of the main component have been derived graphically. Then — also graphically — theoretical values for the number of individuals in the classes common with the minor component have been calculated. By subtracting these class numbers from those actually found, a first isolation of the minor

¹ Here «normally» is used in the meaning of «Gaussian».

component was obtained. By means of graphic levelling a theoretical class number was now calculated in the same way as previously for the main component. Finally the actually distinguished numbers of individuals of the common classes were divided in proportion to the theoretical values. A first decomposition of the material was thus obtained. Mean values and the standard deviation of the components were calculated in the ordinary way.

A second approximation was now undertaken. By the aid of the total numbers of individuals and the distribution constants received, theoretical class values were again calculated for both components, after which the common classes were divided in proportion to these figures. Then again individual numbers, N_0 , N_1 mean values MD_0 , MD_1 , and standard deviation s_0 , s_1 , were calculated from the component distributions thus received. They diverged only slightly from the corresponding figures of the first approximation. Tab. 2 shows the result of the second approximation, which may be considered to give definite values for the parameters sought for.

The result of the decomposition is shown in diagrams 1, 7, 9, 13, 15. The main component seems on the whole to have the shape of a Gaussian curve. The minor component, too, has a rather regular shape but the slight number of individuals make any certain conclusions impossible. In any case it may be said that the material does not contradict the hypothesis of two normally distributed components, yet it must naturally be considered a working hypothesis as long as there is no proof of an actual divergence of the blood cells in the two components.

As we see, Elfving has considered cause to carry out a decomposition in the same 4 cases which already have been decomposed by using Mogensen's method. Elfving has also decomposed case 9, as the skewness calculated according to Charlier and Lindeberg has given reason to suppose that there is a left component in the curve. This left component will, however, be the smallest of all in volume as only 44 cells can be assigned to it. In this connection it should be pointed out that Mogensen's method as regards inversion and drawing of diagram lines based hereupon, give rise to varied interpretations with consequently varied results. When case 9 was decomposed according to Mogensen, no minor component was obtained. In the present decomposition, according to Mogensen, it has been stated that the method is very sensitive and the just mentioned negative result in decomposing case 9 may be attributed just to this quality in Mogensen's method.

If we leave out case 9, the case in which Elfving found the smallest left component, then his classification of the curves based on skewness and the results which he has reached hereby show such a pleasing conformity with the grouping of the curves according to

Mogensen's inverting method and the values obtained hereby, that the methods may be said to confirm each other. On account of these results we have a valuable proof of the investigation being correct from a mathematical point of view.

Comment.

The investigation has shown that the distribution curves in 4 of the 15 cases of tape-worm anemia (1, 7, 13, 15) are the same as in cryptogenetic pernicious anemia, in that they are asymmetrical and divisible into components. In all these 4 cases the anemia was comparatively severe. In the fifth case (9), the one which could be decomposed according to Elfving, but not according to Mogensen, the blood values were decidedly higher. There certainly were 3 cases (2, 4, 11) among the tape-worm anemia cases in which the anemia was very severe, and a decomposition had not been possible, but it may be questioned whether there was not a right component in these curves which outbalanced the left component. The large standard deviation of the curve supports this supposition. There are two cases of cryptogenetic pernicious anemia (No. 4 and 9) in Mogensen's thesis where the case is similar. In the remaining 7 cases (3, 5, 6, 8, 10, 12, 14) the distribution curves come closer to the normal curve on the whole, in the same degree as the blood values increase.

Throughout his material of cryptogenetic pernicious anemia Mogensen found distribution curves which could be decomposed. This may be because the anemia was always severe in his cases. On that account he calls for investigations of the distribution curve in patients with less developed cryptogenetic pernicious anemia. Before such an investigation has been undertaken it cannot be definitely determined whether the distribution curves in tape-worm anemia and cryptogenetic pernicious anemia are quite similar. The result of my investigation indicates, however, that this may be the case.

Summary.

The results of the examination of the Price-Jones' curve in 15 cases of tape-worm anemia were as follows:

1. There was no correlation between the size of the mean diameter and the size of the standard deviation.

2. Neither could a correlation between the size of the mean diameter and the red cell count be stated.

3. On the other hand there was a close agreement between the red cell count and the size of the standard deviation, as the standard deviation was largest in the cases where the red cell counts were the lowest.

4. These results agree exactly with the results that Mogensen and Price-Jones obtained in cryptogenetic pernicious anemia.

5. In 4 cases of tape-worm anemia the curves were clearly asymmetrical and could be divided into one left and one main component as in cryptogenetic pernicious anemia (Mogensen). In 3 further cases, which have fairly symmetrical distribution curves, there is reason to believe that behind the abnormally great deviation is hidden not only a left component but a right component as well. The remaining eight curves are practically symmetrical but the increased mean diameter and standard deviation cause them to differ from the normal curve.

6. The distribution curve in severe tape-worm anemia shows traits which are characteristic of cryptogenetic pernicious anemia.

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Über die klinische Bedeutung der Tuberkulinwiederholungsreaktion.

Von

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(Bei der Redaktion am 29. November 1943 eingegangen).

Einleitung.

Bei der Entscheidung der Frage nach der Aktivität der Lungentuberkulosefälle kommt man dazu, sich in bedeutendem Masse auf so unspezifische Symptome, wie das Auftreten von Fieber und die Ergebnisse der Senkungsreaktion der roten Blutkörperchen, zu stützen. Auch der Röntgenbefund allein vermag nur in einem Teil der Fälle die Frage, ob der betreffende Fall sich im aktiven Stadium befindet, sicher zu beantworten. Sogar das verschärfte Fahren nach Tuberkelbazillen mit Hilfe von Magenspülungen, Züchtungen auf künstlichen Nährböden und Meerschweinchenversuchen liefert namentlich in Fällen von beginnender Lungentuberkulose oftmals ein negatives Resultat. Ein Nachteil der letztgenannten Verfahren ist ferner die lange Zeit, die sie beanspruchen, bevor man zu einem Schluss gelangt. Die serologischen Reaktionen, von denen gegenwärtig die Methoden Meinickes, Besredkas und Witebsky-Klingenstein-Kuhns als die besten gelten, können häufig ein wertvolles Hilfsmittel bei der Diagnostizierung der aktiven Lungentuberkulose ausmachen, doch haben auch sie den Nachteil, dass sie in Fällen von beginnender Lungentuberkulose oft zu einem negativen Ergebnis führen. Falls ein Forscher mit Hilfe irgendeiner Seroreaktion bei aktiver Tuberkulose fast ausnahmslos ein positives Resultat erzielt, liefert die Reaktion dann

auch in solcher Menge unspezifische positive Ergebnisse bei inaktiver Tuberkulose, bei Gesunden und bei Personen mit nicht tuberkulösen Krankheiten, dass der Reaktionswert darunter leidet. Die Grenze zwischen aktiver und inaktiver Tuberkulose ist so undeutlich, dass man wahrscheinlich nie ein Mittel finden wird, welches mit absoluter Gewissheit die Aktivitätsfrage in jedem einzelnen Tuberkulosefall entscheidet. Aber die Übereinstimmung des klinischen Gesamtbildes mit manchem Symptom und Reaktionsbefund nähert uns schon der unbedingten Gewissheit.

Die Tuberkulinreaktion gibt mit wenigen Ausnahmen (z. B. Masern, Scharlach, Typhus, oftmals Tuberkulose im letzten Stadium) die Antwort auf die Frage, ob eine Person mit Tuberkulose angesteckt worden ist oder nicht, aber in bezug auf die Aktivität eines Tuberkulosefalles kann man sich nur bei Kleinkindern mit einer gewissen Wahrscheinlichkeit auf ihre Aussage verlassen.

Dass das Verhältnis zwischen zwei aufeinanderfolgenden Tuberkulinproben für die Aktivitätsdiagnose der Tuberkulosefälle von Bedeutung sei, wird in der Fachliteratur erwähnt, aber die Zahl der untersuchten Fälle ist gering und die Verfahren selbst sind in mancher Beziehung schwankend, weshalb man auf ihrer Grundlage keine sicheren Schlüsse in bezug auf den Wert der Methode ziehen kann. Es ist bemerkenswert, dass diese Frage, wenigstens nach der mir zugänglichen Literatur zu schliessen, sehr wenig studiert worden ist.

Ich habe in meiner Arbeit danach gestrebt, den Wert der wiederholten Tuberkulinreaktion für die Stellung der Aktivitätsdiagnose zu erforschen und die Bedeutung, welche ihr im Vergleich mit einigen anderen Aktivitätssymptomen zukommt, klarzustellen. Gleichzeitig wurden Vergleiche zwischen den Kutan- (Pirquet) und Intrakutanreaktionen (Mantoux) beim Studium der wiederholten Tuberkulinreaktionen angestellt.

Geschichtliches.

Schon v. Pirquet (1907) weist in seiner ersten Veröffentlichung darauf hin, dass in der Tuberkulinprobe die langsamen Reaktionsformen, die erst nach einer Reihe von Tagen ihren Höhepunkt erreichen oder erst nach Wiederholung des Versuchs positiv werden, m. a. W. die sekundären Reaktionen, auf inaktive Tuberkulose

schliessen lassen, während die Frühreaktionen in der Mehrzahl der Fälle auf manifeste Tuberkulose bezogen werden können.

Ellermann und Erlandsen (1909) haben festgestellt, dass Tuberkulinproben, wenn sie zwei Tage nacheinander angestellt werden, keinen Unterschied aufweisen, dass aber schon wenn vier Tage zwischen den Reaktionen liegen, eine deutliche Steigerung vorhanden ist. Noch nach 101 und 137 Tagen ist ein deutliches Steigen nachweisbar. Weiter haben die Forscher konstatiert, dass die Sensibilisierung eine universelle, keine lokale Erscheinung ist.

Kögel (1912) beobachtete in den käsigen Fällen bei guter klinischer Besserung im ganzen einen starken und bei Wiederholung der Reaktion einen steigenden Pirquet; umgekehrt zeigten die käsigen Fälle bei geringer klinischer Besserung und bei Verschlechterung ein Sinken des starken oder ein Gleichbleiben des schwachen Pirquet. Für die fibrösen Fälle ohne Tuberkelbazillen im Sputum ist also eine verspätete und oft geringe Kutanreaktion charakteristisch. In ganz leichten, abgelaufenen Fällen findet im Laufe der Besserung in der Regel kein Stärkerwerden des Pirquet statt. In den fibrösen Fällen mit Tuberkelbazillen im Auswurf und mit aktiven Erscheinungen zeigt sich bei erheblicher Besserung der klinischen Symptome in der Regel ein Stärkerwerden des Pirquet und ein schnelleres Auftreten der Reaktion. Der Forscher glaubt, dass ein wiederholter Pirquet für die Prognose der Lungentuberkulose von Bedeutung sei.

Grundt (1913) studierte an 130 Kranken die Pirquetsche Reaktion mit einmonatigen Pausen vier verschiedene Male und stellte dabei fest, dass die steigende Sensibilität der Fälle, die die günstigste Prognose haben, am stärksten ist, wogegen negatives oder unzweifelhaftes Sinken als ein ungünstiges Zeichen aufzufassen sei.

Auch Pättälä (1943) beobachtete, dass die erneute Pirquetprobe bei Lupösen eine geringere Erweiterung des Flächeninhalts verursachte, als die Wiederholung der Reaktion bei Gesunden.

Unter den Forschern, welche die Wiederholungsreaktion mit Hilfe der intrakutanen Tuberkulinprobe studiert haben, ist als der älteste Rozenblat (1913) zu nennen, der bei seinen Versuchen an 20 Kindern (9 klinisch tuberkulöse, 11 klinisch nicht tuberkulöse Fälle) zu dem Ergebnis kam, dass die intrakutane Wiederholungsreaktion (mit 8-tägigen Pausen) nicht die Frage von der Aktivität eines Falles zu entscheiden vermag.

Zu entgegengesetzten Resultaten führten die Untersuchungen von Bessau und Schwenke (1914). Sie bedienten sich dabei des Alt-Tuberkulins in den Verdünnungen 1: 10000, 1: 1000 und 1: 100 und wiederholten die Reaktion nach 8 Tagen. Die genannten Verfasser bezeichnen den Zuwachs des Reaktionsdurchmessers um 5 mm als fraglich, um 10 mm als deutlich und um 15 mm als stark; aber ein noch grösseres Gewicht als den absoluten Massen der Reaktion möchten sie nach ihrer Erfahrung der Reaktionsqualität, speziell dem Grade der Tastbarkeit und der Rötung, beimessen. Sie untersuchten insgesamt 153 Kinder, von denen 24 stets negativ reagierten. Ihre Untersuchungsergebnisse gehen am deutlichsten aus Tabelle 1 hervor.

Die Verfasser erwähnen, dass eine starke Steigerung der lokalen Tuberkulinempfindlichkeit einen aktiv progredienten tuberkulösen Prozess mit sehr grosser Wahrscheinlichkeit ausschliesse.

Pringsheim (1914) studierte nach Bessau die Wiederholung der Tuberkulinreaktionen an Erwachsenen. Von den 125 untersuchten Fällen war einer tuberkulinnegativ. In einem Teil der Fälle wurde auch die Pirquet-Probe ausgeführt, wobei in 10 % derselben der Parallelismus fehlte. Auch die Ergebnisse Pringsheims sind in Tabelle 1 wiedergegeben.

Marchionini (1929), Reiss (1932) und Cvetkova (1934) haben die intrakutane Tuberkulinwiederholungsreaktion mit achttägigen Zwischenzeiten bei Hauttuberkulose untersucht. Von ihren Resultaten seien folgende erwähnt. Marchionini erhielt in 137 Fällen von aktiver Hauttuberkulose 113 mal (= 82.5 %) die gleiche oder eine abgeschwächte Reaktion, und 24 mal (= 17.5 %) eine Steigerung derselben. Unter 89 Kontrollfällen zeigten 13 die gleiche Reaktion und 76 (= 85.4 %) eine Steigerung. 5 Fälle mit aktiver Tuberkulose anderer Organe lieferten alle die gleiche Reaktion. Von 28 Fällen mit Lupus erythematodes hatten 11 die gleiche Reaktion, 16 Steigerung und 1 Abschwächung. Der Verfasser erwähnt, dass eine Abschwächung der Wiederholungsreaktion bei kachektischen Individuen vorkomme. Reiss untersuchte im ganzen 203 Fälle, darunter 30 % mit manifester Hauttuberkulose (Lupus erythematodes mitgezählt). Von den nicht tuberkulösen Fällen waren 10 % negativ, 43 % zeigten eine gesteigerte, 10 % die gleiche und 37 % eine abgeschwächte Reaktion, welche letztere vom Verfasser kachektische Reaktion genannt wird. In 10 % der inaktiven

Tabelle 1.
Die Ergebnisse der intrakutanen Tuberkulinwiederholungsprobe nach Bessau und Schwenke (1914) sowie Pringsheim (1914).

Autor (Publikations- jahr) u. Gesamt- anzahl der Fälle	Beschaffenheit der Fälle	Anzahl der Fälle		Keine Steigerung		Fragliche Steigerung		Deutliche Steigerung		Starke Steigerung	
		Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%
Bessau u. Schwenke (1914) 129 Fälle	Klinisch gesicherte aktive Tuber- kulosen	13	77.0	10	77.0	2	15.5	1	7.5	—	—
	Klinisch Tuberkulose wahrschein- lich	23	65.0	15	65.0	5	22.0	3	13.0	—	—
	Klinisch Tuberkulose nicht aus- geschlossen	48	42.0	20	42.0	12	25.0	14	29.0	2	4.0
	Klinisch ohne nachweisbare Tu- berkulose	45	4.5	2	4.5	7	15.5	27	60.0	9	20.0
	Offene Lungentuberkulose	41	78.5	32	78.5	3	7.2	5	11.9	1	2.4
Pringsheim (1914) 124 Fälle	Klinisch Tuberkulose wahrschein- lich	30	24.	8	24.	2	6	10	30	10	30
	Klinisch Tuberkulose nicht aus- geschlossen	4	—	—	—	1	25	2	50	1	25
	Klinisch tuberkulosefreie Fälle	48	12.5	5	12.5	—	—	23	45.8	19	41.7

Lungen- und Hilustuberkulosefälle war die Reaktion negativ, in 53 % gesteigert und in 37 % kachektisch. Bei manifester Hauttuberkulose fand man in 21 % der Fälle eine gesteigerte, in 50 % die gleiche und in 29 % eine kachektische Reaktion. Für Lupus erythematodes wurden die Prozentsätze 69,3, 7,7 und 23 gefunden. Cvetkova untersuchte im ganzen 174 Kranke mit verschiedenen Formen der Hauttuberkulose und erhielt dabei eine Reaktion des aktiven Typus in 61,6 %; des inaktiven Typus in 9,3 % und des kachektischen Typus in 29,1 % der Fälle. Die Verfasserin erwähnt, dass die Reaktion nach Bessau keine Schlussfolgerungen hinsichtlich der Prognose und des Immunitätszustandes ziehen lässt.

BJÖRNSTAD (1941) hat Untersuchungen über die durch die intrakutane Tuberkulinreaktion hervorgerufene Sensibilisierung ausgeführt und beobachtet, dass die Sensibilisierung bleibt lange Zeit, ja sogar monatelang bestehen. Verf. beobachtete eine Sensibilisierung sowohl bei Patienten mit aktiver Hauttuberkulose als auch bei Patienten ohne nachweisbare Tuberkulose.

Lobban (1930) studierte die Bedeutung von nach einem Monat oder mehr wiederholten intrakutanen Tuberkulinreaktionen für die Prognose der Tuberkulosefälle und fand bei Besserung ein Steigen der Tuberkulinempfindlichkeit, bei Verschlechterung ein Sinken derselben, während die Empfindlichkeit in stationären Fällen unverändert blieb.

Forbes (1939) bewerkstelligte an gesunden Personen (35 Studenten) in Zwischenräumen von 1, 2, 3, 4 und 6 Wochen intrakutane Tuberkulinprüfungen. Die Resultate zeigten das fast konstante Auftreten einer Überempfindlichkeit nach der ersten Probe, wenn sie nach einer kürzeren Zwischenzeit als 3 Wochen wiederholt wurde.

Material.

Die vorliegenden Untersuchungen wurden an Kranken und Personal aus zwei finnischen Kriegslazaretten sowie an Soldaten einer Ausbildungszentralstelle ausgeführt.¹ Mit Ausnahme derje-

¹ In diesem Zusammenhang habe ich das Vergnügen, den Chefärzten der betreffenden Kriegslazarette, den Krankenschwestern sowie allen Militär- und Zivilpersonen, die mir in der einen oder anderen Weise bei der Ausführung und Veröffentlichung der vorliegenden Arbeit behilflich gewesen sind, meine aufrichtige Dankbarkeit zu bezeugen.

Tabelle 2.

Übersicht über das mit den Wiederholungsreaktionen nach Mantoux und Pirquet untersuchte Material.

Art der Fälle	Untersuchungs- methode		Zusam- men
	Mantoux	Pirquet	
	Anzahl	Anzahl	
Tuberculosis pulmonum I	46	21	67
Tuberculosis pulmonum II	50	33	83
Tuberculosis pulmonum III	59	46	105
Tub. pulm. zusammen	155	100	255
Tub. inactiva	12	13	25
Pleuritis exsudativa	48	15	63
Sonstige Tuberkulose	2	6	8
Suspekte Tuberkulose	9	5	14
Andere Krankheiten	9	13	22
Gesunde	83	195	278
BCG-Geimpfte	67	49	116
Gesamtzahl der Fälle	385	396	781

nigen Personen, die bei den nach beiden Intervallen vollzogenen Tuberkulinprüfungen negativ reagierten, wurden insgesamt 781 Personen untersucht, und zwar kam in 385 Fällen die Mantoux- und in 396 Fällen die Pirquet-Methode zur Anwendung. Eine Übersicht über die Beschaffenheit und Verteilung des untersuchten Materials in bezug auf die Mantoux- und die Pirquet-Proben findet sich in Tabelle 2.

In der obengenannten Ausbildungszentrale fanden die Tuberkulinprüfungen der Rekruten im Spätwinter 1943 statt. In Verbindung damit wurden die Tuberkulinnegativen mit BCG geimpft, und 116 derselben wurden späterhin ebenfalls in die vorliegende Untersuchung hineinbezogen.

Technik.

Zur Ausführung der Mantoux- wie auch der Pirquet-Prüfungen benutzte ich das Alt-Tuberkulin (Präparat der Behring-Werke). Für die Mantoux-Reaktionen diente eine Grundverdünnungslösung 1: 10, aus welcher jedes Mal die Verdünnung 1: 1000 her-

gestellt wurde. Bei allen Mantoux-Versuchen kam die gleiche (1:1000) Verdünnung zur Anwendung. Wenigstens nach meiner Erfahrung ist es unnötig, mit stärkeren Verdünnungen zu beginnen, denn keine der von mir untersuchten Personen reagierte irgendwie ernster auf die erhaltenen Tuberkulinmengen. Nach Reinigung der Haut mit Äther wurde beim ersten Versuch je 0.1 cm³ an der Volarseite beider Unterarme, etwa 10 cm proximalwärts des Handgelenks, intrakutan eingespritzt. Bei der späteren Prüfung erfolgten die Injektionen 10 cm proximalwärts der vorigen Injektionsstelle.

Die Pirquet-Proben fanden in üblicher Weise, nachdem die Haut mit Äther gereinigt worden war, mit Hilfe des Impfbohrers statt. Zu der ersten Probe und zu der Wiederholungsreaktion benutzte ich für dieselbe Person immer dieselbe Tuberkulinverpackung. Die Bohrungen wurden an denselben Stellen am Unterarm wie die Mantoux-Prüfungen bewerkstelligt.

Um die Resultate der Tuberkulinprüfungen zu beurteilen, wurden 24 und 48 Std. nach Ausführung des Versuchs zwei winkelrecht zu einander gestellte Durchmesser der Rötung gemessen. Ein eventuell auftretender, mit der lymphangitischen Rötung zusammenhängender Streifen blieb jedoch bei der Messung unberücksichtigt. Die Messungen wurden von Krankenschwestern oder Sanitätsunteroffizieren ausgeführt. Um die Technik zu vereinfachen und etwaige subjektive Deutungen zu vermindern, berücksichtigte man bei der Beurteilung der Resultate weder die Intensität der Rötung, noch die eventuellen Verschiedenheiten der Infiltratkonsistenz. An beiden Unterarmen einzeln wurde von den winkelrechten Rötungsdurchmessern der Durchschnittswert ausgerechnet und die beiden Mittelwerte dann zusammengezählt, so dass bei der Beurteilung der Tuberkulinreaktionen auf jeden 24-Std.- und jeden 48-Std.-Wert eine Ziffer entfällt; für den Wiederholungsversuch gilt dasselbe. Darauf wurde der Unterschied für die 24- und die 48-Std.-Werte ausgerechnet. Der gefundene Unterschied wurde noch in Prozenten bestimmt, weil bei den Mantoux-Reaktionen stets die gleiche Tuberkulinmenge zur Anwendung kam, wodurch die in verschiedenem Grade tuberkulinempfindlichen Individuen miteinander vergleichbar werden. Nachstehend geben wir als Beispiel die Messungsergebnisse und Ausrechnungen von Fall 553 wieder.

	Mantoux I		Mantoux II		I		II	
	24 Std.	48 Std.	24 Std.	48 Std.	24 Std.	48 Std.	24 Std.	48 Std.
Rechts; ..	26 × 23	50 × 50	28 × 35	45 × 37	24.5	50	46.5	41
Links; ..	32 × 30	70 × 50	54 × 50	35 × 30	31	60	52	32.5
					55.5 mm	110 mm	98.5 mm	73.5 mm

II—I	II—I	II—I	II—I
24 Std.	48 Std.	24 Std.	48 Std.
+43mm	—36.5mm	+77.5 %	—33.2 %

Die Resultate der Pirquet-Reaktionen wurden nach demselben Messungsverfahren und derselben Bestimmungsweise des Unterschieds zwischen der ersten und der wiederholten Prüfung wie bei den Mantoux-Reaktionen beurteilt.

Es wäre eine andere Möglichkeit, die Unterschiede der mittleren Durchmesser an und für sich zu beurteilen, wie es z. B. Bessau und Schwenke getan; sie benutzten jedoch als erste Tuberkulinverdünnung die Lösung 1:10000, darauf 1:1000 und bei Bedarf 1:100. Von diesen Verdünnungen kam dann bei der Wiederholungsprüfung die kleinste Tuberkulinmenge, die gerade noch eine positive Reaktion geliefert hatte, zur Anwendung. Auf diese Art erhielten die genannten Forscher bei ihren Prüfungen wahrscheinlich besser als ich Rötungen der gleichen Grössenklasse.

Eine dritte Methode wäre natürlich, die Flächenräume der Rötungen in den Erst- und Zweitproben miteinander zu vergleichen. Da aber die Wirkung der Messungsfehler der Durchmesser auf den Vergleich der Ergebnisse zunimmt, habe ich mich mit dem einfacheren Durchmesservergleich begnügt.

Methodik.

Es gibt drei Möglichkeiten für die Resultate der Tuberkulinwiederholungsreaktion. Erstens kann eine Person auf einen wiederholten, gleich grossen Tuberkulinreiz schwächer als auf den ersten Reiz reagieren. Ein solches Verhalten habe ich Abschwächung

Tabelle 3.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 10 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	46	16	35	9	19	21	46
Tub. pulm. II	50	20	40	6	12	24	48
Tub. pulm. III	59	24	41	12	20	23	39
Tub. pulm. zusammen ..	155	60	38.7	27	17.4	68	43.9
Tub. inactiva	12	1		—		11	
Pleuritis exsudativa	48	2	4	4	8	42	88
Sonstige Tuberkulose	2	1		—		1	
Suspekte Tuberkulose	9	2		—		7	
Andere Krankheiten	9	1		—		8	
Gesunde	83	8	9.6	4	4.8	71	85.6
BCG-Geimpfte	67	38	56.7	4	6.0	25	37.3
Zusammen	385						

genannt. Die zweite Möglichkeit ist die, dass beide Resultate die gleichen sind. Drittens kann der erste Tuberkulinreiz den Organismus so empfindlich machen, dass die erneute Injektion einer gleich grossen Tuberkulinmenge eine stärkere Reaktion hervorruft, wobei auch die Rötung der Impfgegend sich ausdehnt. Es wäre natürlich gut, auch die Intensität der aufgetretenen Rötung möglichst objektiv beurteilen zu können. Mitunter macht sich die Überempfindlichkeit nur in dieser Weise bemerkbar. Mit den etwaigen Verschiedenheiten der Infiltratkonsistenz verhält es sich ungefähr ebenso, doch würde die Berücksichtigung aller dieser Faktoren wie auch die Beachtung eventueller Unterschiede bei den Bläschen die Beurteilung der Resultate verwickelter machen und die Möglichkeit subjektiver Deutungen erhöhen. Damit würde sich auch in dieser Hinsicht der praktische Wert der Methode vermindern.

Wenn man die auf oben beispielsweise angeführte Art gewonnenen Prozentzahlen als Beurteilungsgrundlage benutzt, hat man

Tabelle 4.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

20 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	46	12	26	18	39	16	35
Tub. pulm. II	50	16	32	17	34	17	34
Tub. pulm. III	59	19	32	21	36	19	32
Tub. pulm. zusammen ..	155	47	30.3	56	36.1	52	33.6
Tub. inactiva	12	—		2		10	
Pleuritis exsudativa	48	2	4	12	25	34	71
Sonstige Tuberkulose	2	1		1		—	
Suspekte Tuberkulose	9	2		3		4	
Andere Krankheiten	9	1		1		7	
Gesunde	83	6	7.2	8	9.6	69	83.2
BCG-Geimpfte	67	33	49.3	11	16.4	23	34.3
Zusammen	385						

die Frage zu entscheiden, wann das Ergebnis der Wiederholungsreaktion mit demjenigen der ersten Tuberkulinprobe als gleich betrachtet werden kann. Am einfachsten wäre es natürlich, die Resultate nur dann als übereinstimmend zu bezeichnen, wenn sie sich auch mathematisch decken; dann würde aber die Gruppe so klein werden, dass sie jede nennenswerte praktische Bedeutung verliert; ausserdem wäre die biologische Berechtigung eines so strengen Verhaltens zum mindesten fraglich. Um diese Frage zu entscheiden, gruppierte ich die gefundenen Resultate in Tabellen derart, dass Tabelle 3 die Fälle umfasst, wo das aus der Mantoux-Wiederholungsreaktion hervorgehende Resultat in betreff der 48-Std.-Werte als das gleiche betrachtet wurde, sofern der Unterschied zwischen der ersten und der erneuten Prüfung nicht 10 % erreichte. In Tabelle 4 fand die Gruppierung in der Weise statt, dass das Resultat der 48-Std.-Werte für übereinstimmend galt, falls der Unterschied zwischen der ersten und der erneuten Reaktion nicht volle 20 % ausmachte. In Tabelle 5 schliesslich wurde das

Tabelle 5.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 30 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	46	9	19	27	59	10	22
Tub. pulm. II	50	13	26	25	50	12	24
Tub. pulm. III	59	13	22	29	49	17	29
Tub. pulm. zusammen ..	155	35	22.6	81	52.2	39	25.2
Tub. inactiva	12	—	—	4		8	
Pleuritis exsudativa	48	2	4	17	36	29	60
Sonstige Tuberkulose	2	1		1		—	
Suspekte Tuberkulose	9	2		4		3	
Andere Krankheiten	9	—		2		7	
Gesunde	83	5	6.0	18	21.7	60	72.3
BCG-Geimpfte	67	26	38.8	21	31.3	20	29.9
Zusammen	385						

Gleichheitszeichen dann gesetzt, wenn der besagte Prozentsatz geringer war als 30.

Wenn man die Resultate der Tabellen 3, 4 und 5 näher betrachtet, geht aus ihnen allen unzweideutig hervor, dass Abschwächung und Gleichbleiben für aktive Tuberkulose sprechen, während Steigerung bei Gesunden häufiger vorkommt. Gibt man zuerst acht auf das Auftreten der Sensibilitätssteigerung bei Lungenkranken und bei Gesunden, so wird in Tabelle 3 schon ein schwaches (10 %) Steigen mitgezählt, in Tabelle 4 ein stärkeres (20 %) und in Tabelle 5 ein noch stärkeres (30 %). Das Auftreten der Steigerung laut Tabelle 3, 4 und 5 bei Lungenkranken und Gesunden, in Prozenten ausgedrückt, ergibt die Zusammenstellung 1.

Zusammenstellung 1.

Mit Berücksichtigung

	schwacher (10 %) Steigerung	mässiger (20 %) Steigerung	stärkerer (30 %) Steigerung
Tub.pulm.	43.9 %	33.6 %	25.2 %
Gesunde	85.6 %	83.2 %	72.3 %

Wie aus der obigen Zusammenstellung ersichtlich, lässt sich eine schwache Sensibilitätssteigerung verhältnismässig oft auch in Fällen von Lungentuberkulose beobachten, aber je stärker die Steigerung ist, um so bestimmter spricht sie gegen aktive Tuberkulose.

Studiert man nun das Vorkommen der Abschwächung bei Lungenkranken und Gesunden, so ergibt sich in obenbeschriebener Weise die Zusammenstellung 2.

Zusammenstellung 2.

Mit Berücksichtigung

	starker (30 %) Abschwächung allein	auch mässiger (20 %) Abschwächung	auch schwacher (10 %) Abschwächung
Tub.pulm.	22.6 %	30.3 %	38.7 %
Gesunde.....	6.0 %	7.2 %	9.6 %

Die Zusammenstellung 2 zeigt, dass schon eine geringe Abschwächung auf Lungentuberkulose bezogen werden kann, aber dass eine stärkere Hemmung nicht nennenswert spezifischer auf aktive Tuberkulose hindeutet.

Werden nun Abschwächung und gleiches Resultat zusammengezählt, so kommt man in der entsprechenden Weise zur Zusammenstellung 3.

Zusammenstellung 3.

Mit Berücksichtigung

	von Hemmung u. genauer Übereinstimmung (Tab. 3)	von Hemmung u. 20 %iger Übereinstimmung (Tab. 4)	von Hemmung u. 30 %iger Übereinstimmung (Tab. 5)
Tub.pulm.....	56.1 %	66.4 %	74.8 %
Gesunde.....	14.4 %	16.8 %	27.7 %

Aus Zusammenstellung 3 geht hervor, dass mit Beachtung der Hemmung und des gleichen Resultats zusammen das in Tabelle 4 vorgelegte Verfahren sich für diagnostische Zwecke am besten eignet, denn Übereinstimmung des Resultats und Hemmung zusammen findet man fast viermal so oft bei aktiver Lungentuberkulose, als bei Gesunden.

Die Tabellen 6, 7 und 8 enthalten die Ergebnisse der Pirquet-Wiederholungsreaktion, in derselben Weise gruppiert.

Tabelle 6.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 10 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	21	13	62	4	19	4	19
Tub. pulm. II	33	16	49	5	15	12	36
Tub. pulm. III	46	21	46	6	13	19	41
Tub. pulm. zusammen ..	100	50	50.0	15	15.0	35	35.0
Tub. inactiva	13	2		1		10	
Pleuritis exsudativa	15	6		—		9	
Sonstige Tuberkulose	6	3		2		1	
Suspekte Tuberkulose	5	—		3		2	
Andere Krankheiten	13	1		1		11	
Gesunde	195	85	43.6	33	16.9	77	39.5
BCG-Geimpfte	49	17	35	5	10	27	55
Zusammen	396						

Wie aus den Tabellen ersichtlich, ist Sensibilitätssteigerung eine etwas häufigere Erscheinung bei Gesunden, als in Fällen von aktiver Lungentuberkulose, — nach welcher der drei obenbeschriebenen Methoden man auch die Gleichheit des Resultats bestimmen möge. Der Unterschied ist indessen so gering, dass die Pirquet-Wiederholungsreaktion, wenn die 48-Std.-Werte als Beurteilungsgrundlage dienen, keine Rolle in der Diagnostik der aktiven Lungentuberkulose zu spielen scheint.

Man kommt also zu dem Schluss, dass bei der *Mantoux-Wiederholungsreaktion* die 48 Std. nach der Tuberkulinprüfung abgelesenen Resultate dann am ehesten für ein gleiches Ergebnis angesehen werden können, wenn die Summen der Mittelwerte zweier winkelrechter Rötungsdurchmesser einen Unterschied zwischen Erst- und Wiederholungsreaktion aufweisen, der nicht 20 % erreicht. Zugleich ergibt es sich, dass eine starke Steigerung der Sensibilität besonders charakteristisch für Gesunde ist, wogegen ein übereinstimmendes Resultat

Tabelle 7.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	21	12	57	6	29	3	14
Tub. pulm. II	33	12	36	14	43	7	21
Tub. pulm. III	46	17	37	11	24	18	39
Tub. pulm. zusammen ..	100	41	41.0	31	31.0	28	28.0
Tub. inactiva	13	1		3		9	
Pleuritis exsudativa	15	4		2		9	
Sonstige Tuberkulose	6	2		4		—	
Suspekte Tuberkulose	5	—		3		2	
Andere Krankheiten	13	—		3		10	
Gesunde	195	65	33.3	69	35.4	61	31.3
BCG-Geimpfte	49	10	20	13	27	26	53
Zusammen	396						

und schon eine geringe Hemmung häufig die Fälle von aktiver Lungentuberkulose auszeichnet.

Soviel ich weiss, hat noch kein Forscher versucht, die Frage zu entscheiden, welche Zwischenzeit für die Ausführung der Tuberkulinwiederholungsprüfung am günstigsten wäre. Ellermann und Erlandsen erwähnen freilich, dass bei wiederholter Tuberkulinprüfung derselben Person Überempfindlichkeit sich schon einstelle, wenn 4 Tage zwischen den Reaktionen liegen, und dass noch 137 Tage später eine deutliche Steigerung nachzuweisen sei. Der Wert der Tuberkulinwiederholungsreaktion wäre praktisch grösser, falls die in Frage stehende Pause möglichst kurz wäre. Infolgedessen und zweitens deshalb, weil meine Untersuchungen sich zum Teil auf Begutachtungskranke bezogen, die wegen des starken Platzmangels im Lazarett möglichst bald die Beobachtungsabteilung verlassen sollten, wählte ich 5 Tage als Intervall der Tuberkulinwiederholungsreaktionen. Um darüber ins klare zu kommen, ob

Tabelle 8.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und erneuten Prüfung ergaben, der nicht 30 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	21	8	38	10	48	3	14
Tub. pulm. II	33	5	15	22	67	6	18
Tub. pulm. III	46	11	24	19	41	16	35
Tub. pulm. zusammen ..	100	24	24.0	51	51.0	25	25.0
Tub. inactiva	13	—		7		6	
Pleuritis exsudativa	15	3		3		9	
Sonstige Tuberkulose	6	2		4		—	
Suspekte Tuberkulose	5	—		3		2	
Andere Krankheiten	13	—		5		8	
Gesunde	195	52	26.7	91	46.6	52	26.7
BCG-Geimpfte	49	7	14	19	39	23	47
Zusammen	396						

irgendein anderer Zwischenraum günstiger gewesen wäre, erneute ich meine Prüfung bei einem Teil des Materials nach 14 Tagen und bei einem Teil der gesunden Wehrpflichtigen nach einem Monat. Die Zahl der letztgenannten Gesunden war anfänglich über 200, doch wurden die meisten von ihnen nach der ersten Tuberkulinprüfung dienstlich abkommandiert; nur 34 Rekruten blieben zurück und nahmen an der Prüfung nach einem Monat teil.

Die auf 48 Stunden bezüglichen Resultate der Mantoux-Wiederholungsreaktion, als Gleichbleiben betrachtet, sofern der prozentuale Unterschied nicht 20 erreichte, und in drei Gruppen eingeteilt, je nachdem, ob die Zwischenzeit 1 Monat, 14 Tage oder 5 Tage betrug, sind in Tabelle 9 eingetragen.

Bei der Betrachtung der in Tab. 9 wiedergegebenen Resultate wird man in der Gruppe der Lungentuberkulosefälle darauf aufmerksam, dass bei einer Zwischenzeit von 14 Tagen die Sensibilität nur in 15 % der Fälle zunahm. Zwar kam auch bei den Gesunden

Tabelle 9.

Ergebnisse der Mantoux-Wiederholungsreaktion bei Verwendung verschieden langer Zwischenzeiten, wenn die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Inter- vall	Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
			der Sensibilität					
			An- zahl	%	An- zahl	%	An- zahl	%
1 Monat	Gesunde	13	1	8	—		12	92
14 Tage	Tub. pulm. I	10	4		4		2	
	Tub. pulm. II	8	4		4		—	
	Tub. pulm. III	15	9		3		3	
	Tub. pulm. zusammen ..	33	17	52	11	33	5	15
	Pleuritis exsudativa	1	1		—		—	
	Sonstige Tuberkulose	1	1		—		—	
	Suspekte Tuberkulose ..	1	—		1		—	
	Andere Krankheiten	1	1		—		—	
	Gesunde	38	5	13	4	11	29	76
	Zusammen	75						
5 Tage	Tub. pulm. I	36	8		14		14	
	Tub. pulm. II	42	12		13		17	
	Tub. pulm. III	44	10		18		16	
	Tub. pulm. zusammen ..	122	30	25	45	37	47	38
	Tub. inactiva	12	—		2		10	
	Pleuritis exsudativa	47	1		12		34	
	Sonstige Tuberkulose	1	—		1		—	
	Suspekte Tuberkulose ..	8	2		2		4	
	Andere Krankheiten	8	—		1		7	
	Gesunde	32	—		4	12.5	28	87.5
	BCG-Geimpfte	67	33		11		23	
	Zusammen	297						

eine Zunahme der Sensibilität nur in 76 % vor; also ist die Steigerung der Sensibilität um 11.5 % von dem Wert, den sie bei der Zwischenzeit von 5 Tagen aufwies, gesunken. Indessen deutet der Sachverhalt, dass in den Lungentuberkulosefällen die Sensibilitätssteigerung 15 % nach 14 Tagen und 38 % nach 5 Tagen war, darauf hin, dass es vielleicht besser gewesen wäre, ein längeres Intervall als 5 Tage zu wählen. Die Gruppe der Gesunden, wo die Zwischenzeit 1 Monat ausmachte, ist sehr klein, doch bringt das Resultat trotzdem die Richtung zum Ausdruck, dass sich bei Gesunden in der Mantoux-Wiederholungsprüfung sogar noch 1 Monat nach der ersten Probe im allgemeinen eine Sensibilitätszunahme erkennen lässt.

Die Ergebnisse der Mantoux-Wiederholungsreaktion bei Benutzung verschieden langer Intervalle, wenn nur der Fall als Gleichbleiben bezeichnet wurde, wenn nach 48 Stunden die summierten Mittelwerte der für beide Unterarme ausgerechneten zwei winkelfrechten Rötungsdurchmesser in der ersten und der erneuten Probe einen Unterschied von weniger als 10 % aufwiesen, sind aus Tabelle 10 zu ersehen. Eine Prüfung der Resultate ergibt, wenn es sich um ein vierzehntägiges Intervall handelt, dass die zunehmende Sensibilität darlegenden Lungentuberkulosefälle prozentuell von 15 auf 24 gestiegen sind, was bei den Gesunden dem Verhältnis 76 zu 79 entspricht. Vergleicht man die Resultate bei fünftägigem Intervall in den Tabellen 9 u. 10, so findet man, dass die in Prozenten ausgedrückte Sensibilität in den Fällen von Lungentuberkulose von 38 auf 49, bei den Gesunden von 87.5 auf 91 gestiegen ist. Was das Auftreten der Hemmung bei Lungentuberkulose anbelangt, ist zu erwähnen, dass ihr Prozentsatz bei einer Zwischenzeit von 14 Tagen von 52 auf 67 zugenommen hat. Diese Beobachtung zeigt deutlich die Richtung an, dass sogar eine schwache Hemmung recht stark für aktive Tuberkulose spricht.

In Tabelle 11 finden sich die Ergebnisse der Mantoux-Wiederholungsreaktion bei Benutzung verschieden langer Zwischenzeiten; hierbei bezeichnet Gleichbleiben den Fall, dass der Unterschied zwischen dem Resultat der ersten und der erneuten Probe geringer ist als 30 %.

Im Vergleich mit den in Tabelle 9 mitgeteilten Ergebnissen, wo der Fall, dass der besagte Unterschied noch nicht 20 % erreichte, als Gleichbleiben betrachtet wurde, hatte das Prozent der Sensibili-

Tabelle 10.

Ergebnisse der Mantoux-Wiederholungsreaktion bei Verwendung verschieden langer Zwischenzeiten, wenn die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 10 % erreichte.

Inter- vall	Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
			der Sensibilität					
			An- zahl	%	An- zahl	%	An- zahl	%
1 Monat	Gesunde	13	1	8	—		12	92
14 Tage	Tub. pulm. I	10	6		2		2	
	Tub. pulm. II	8	5		—		3	
	Tub. pulm. III	15	11		1		3	
	Tub. pulm. zusammen ..	33	22	67	3	9	8	24
	Pleuritis exsudativa	1	1		—		—	—
	Sonstige Tuberkulose	1	1		—		—	—
	Suspekte Tuberkulose ..	1	—		—		1	
	Andere Krankheiten	1	1		—		—	—
	Gesunde	38	5	13	3	8	30	79
	Zusammen	75						
5 Tage	Tub. pulm. I	36	10		7		19	
	Tub. pulm. II	42	15		6		21	
	Tub. pulm. III	44	13		11		20	
	Tub. pulm. zusammen ..	122	38	31	24	20	60	49
	Tub. inactiva	12	1		—		11	
	Pleuritis exsudativa	47	1		4		42	
	Sonstige Tuberkulose	1	—		—		1	
	Suspekte Tuberkulose ..	8	2		—		6	
	Andere Krankheiten	8	—		—		8	
	Gesunde	32	2	6	1	3	29	91
	BCG-Geimpfte	67	38		4		25	
	Zusammen	297						

Tabelle 11.

Ergebnisse der Mantoux-Wiederholungsreaktion bei Verwendung verschieden langer Zwischenzeiten, wenn die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 30 % erreichte.

Inter- vall	Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
			der Sensibilität					
			An- zahl	%	An- zahl	%	An- zahl	%
1 Monat	Gesunde	13	1	8	—		12	92
14 Tage	Tub. pulm. I	10	3		6		1	
	Tub. pulm. II	8	4		4		—	
	Tub. pulm. III	15	6		6		3	
	Tub. pulm. zusammen ..	33	13	39	16	49	4	12
	Pleuritis exsudativa	1	1		—		—	
	Sonstige Tuberkulose	1	1		—		—	
	Suspekte Tuberkulose ..	1	—		1		—	
	Andere Krankheiten	1	—		1		—	
	Gesunde	38	4	11	8	21	26	68
	Zusammen	75						
5 Tage	Tub. pulm. I	36	6		21		9	
	Tub. pulm. II	42	9		21		12	
	Tub. pulm. III	44	7		23		14	
	Tub. pulm. zusammen ..	122	22	18	65	53	35	29
	Tub. inactiva	12	—		4		8	
	Pleuritis exsudativa	47	1		17		29	
	Sonstige Tuberkulose	1	—		1		—	
	Suspekte Tuberkulose ..	8	2		3		3	
	Andere Krankheiten	8	—		1		7	
	Gesunde	32	—	—	10	31	22	69
BCG-Geimpfte	67	26		21		20		
Zusammen	297							

Tabelle 12.

Ergebnisse der Pirquet-Wiederholungsreaktion bei Verwendung verschieden langer Zwischenzeiten, wenn die nach 48 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Inter- vall	Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
			der Sensibilität					
			An- zahl	%	An- zahl	%	An- zahl	%
1 Monat	Gesunde	21	3	14.5	3	14.5	15	71
14 Tage	Tub. pulm. I	3	2		1		—	
	Tub. pulm. II	5	3		2		—	
	Tub. pulm. III	15	5		4		6	
	Tub. pulm. zusammen ..	23	10	44	7	30	6	26
	Tub. inactiva	1	—		—		1	
	Sonstige Tuberkulose	2	1		1		—	
	Suspekte Tuberkulose ..	3	—		1		2	
	Andere Krankheiten	1	—		—		1	
	Gesunde	32	4	13	11	34	17	53
	Zusammen	62						
5 Tage	Tub. pulm. I	18	10		5		3	
	Tub. pulm. II	28	9		12		7	
	Tub. pulm. III	31	12		7		12	
	Tub. pulm. zusammen ..	77	31	40	24	31	22	29
	Tub. inactiva	12	1		3		8	
	Pleuritis exsudativa	15	4		2		9	
	Sonstige Tuberkulose	4	1		3		—	
	Suspekte Tuberkulose ..	2	—		2		—	
	Andere Krankheiten	12	—		3		9	
	Gesunde	142	58	40.8	55	38.7	29	20.5
	BCG-Geimpfte	49	10	20	13	27	26	53
	Zusammen	313						

tätssteigerung bei einer Zwischenzeit von 14 Tagen bei Lungenkranken von 15 bis 12 und bei Gesunden von 76 bis 68 abgenommen. Vergleicht man in gleicher Weise die Resultate bei fünf-tägigem Intervall, so zeigt es sich, dass die Steigerung bei Lungenkranken prozentuell von 38 bis 29 und bei Gesunden von 87.5 bis 69 gesunken ist. Damit kann auch mit Berücksichtigung verschiedenen langer Intervalle festgestellt werden, dass im Mantoux-Wiederholungsversuch — wenn das Resultat als Gleichbleiben galt, sofern die summierten Mittelwerte der nach 48 Stunden für beide Unterarme bestimmten zwei winkelrechten Rötungsdurchmesser einen geringeren Unterschied als 30 % zwischen der ersten und der erneuten Prüfung aufwiesen — die Prozentziffern der Steigerung bei Lungenkranken und Gesunden sich in der Weise unvorteilhaft einander genähert haben, dass sie bei den Gesunden mehr gesunken sind als bei den Lungenkranken, woraus der Schluss gezogen werden kann, dass es vorteilhafter ist, das Resultat dann als Gleichbleiben zu betrachten, wenn der erwähnte Summenunterschied nicht 20 % erreicht.

Die Ergebnisse der erneuten Pirquet-Reaktion bei verschiedenen langen Intervallen — wobei das Resultat nur dann als Gleichbleiben galt, wenn die nach 48 Stunden für beide Unterarme ausgerechneten Mittelwerte zweier winkelrechter Rötungsdurchmesser einen Summenunterschied von weniger als 20 % bei der ersten und der erneuten Prüfung ergaben — wird in Tabelle 12 veranschaulicht.

Von den Resultaten sei erwähnt, dass bei 21 Gesunden, die nach 1 Monat der erneuten Pirquet-Prüfung unterzogen wurden, 15mal, d. h. in 71 % der Fälle, die Sensibilität gesteigert erschien. Nach einer Zwischenzeit von 14 Tagen war bei 17 von 32 Gesunden, d.h. 53 %, und bei 6 von 23 Lungenkranken, d.h. 26 %, eine Steigerung wahrzunehmen. Betrug das Intervall nur 5 Tage, so hatten von 142 Gesunden nur 29, d. h. 20.5 %, Steigerung, während die Lungentuberkulosefälle ein entgegengesetztes Verhalten zeigten, da in 22 von 77 Fällen, d.h. 28.6 %, Steigerung vorkam. Die Resultate gehen also deutlich dahin, dass in der erneuten Pirquet-Reaktion, wenn die 48-Std.-Werte als Beurteilungsgrundlage dienen, 5 Tage eine zu kurze Zwischenzeit sind, wogegen 14 Tage deutlich bessere Resultate liefern. Die Gruppe der Gesunden, wo das Intervall 1 Monat ausmachte, war sehr klein; doch deutet sie

Tabelle 13.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20% erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	46	5	11	15	33	26	56
Tub. pulm. II	50	7	14	13	26	30	60
Tub. pulm. III	59	8	13	30	51	21	36
Tub. pulm. zusammen ..	155	20	12.9	58	37.4	77	49.7
Tub. inactiva	12	1		2		9	
Pleuritis exsudativa	48	—	—	14	29	34	71
Sonstige Tuberkulose	2	1		1		—	
Suspekte Tuberkulose	9	2		2		5	
Andere Krankheiten	9	—		2		7	
Gesunde	83	2	2.4	5	6.0	76	91.6
BCG-Geimpfte	67	3	4.5	10	14.9	54	80.6
Zusammen	385						

darauf hin, dass man bei den meisten Gesunden Steigerung findet, wenn ein Monat als Intervall dient.

Von meinen Untersuchungen über die beste Zwischenzeit bei der erneuten Tuberkulinreaktion kann zusammenfassend gesagt werden, dass die Mantoux-Resultate gewissermassen darauf hindeuten, dass ein längeres Intervall als 5 Tage möglicherweise günstiger gewesen wäre.

Bei der Pirquet-Wiederholungsprüfung ergab es sich bei Benutzung der 48-Std.-Werte, dass 5 Tage eine zu kurze Zwischenzeit waren, wogegen ein längeres Intervall offenbar bessere Resultate lieferte.

Wenn man im Folgenden die Beurteilungsmöglichkeiten der Resultate studiert, kann die Frage aufgestellt werden, ob es vorteilhafter ist, die Werte von 48 oder 24 Stunden zu benutzen. Die auf 24-Std.-Werten fussenden Ergebnisse der Mantoux-Wiederholungsreaktion, wobei das Resultat als Gleichbleiben betrachtet wurde, wenn der Unterschied der in obenbeschriebener Weise ausgerechneten Mittelwerte weniger als 20 betrug, finden sich in Tabelle 13.

Tabelle 14.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 10 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I	46	8	17	9	20	29	63
Tub. pulm. II	50	10	20	6	12	34	68
Tub. pulm. III	59	13	22	14	24	32	54
Tub. pulm. zusammen ..	155	31	20.0	29	18.7	95	61.3
Tub. inactiva	12	1		1		10	
Pleuritis exsudativa	48	1	2	6	12.5	41	85.5
Sonstige Tuberkulose	2	1		—		1	
Suspekte Tuberkulose	9	2		—		7	
Andere Krankheiten	9	—		1		8	
Gesunde	83	3	3.6	1	1.2	79	95.2
BCG-Geimpfte	67	7	10.5	5	7.4	55	82.1
Zusammen	385						

Tabelle 15.

Ergebnisse der Mantoux-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 30 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	46	2	4	26	57	18	39
Tub. pulm. II	50	2	4	25	50	23	46
Tub. pulm. III	59	7	12	34	58	18	30
Tub. pulm. zusammen ..	155	11	7.1	85	54.8	59	38.1
Tub. inactiva	12	—		3		9	
Pleuritis exsudativa	48	—		19	40	29	60
Sonstige Tuberkulose	2	1		1		—	
Suspekte Tuberkulose	9	1		3		5	
Andere Krankheiten	9	—		3		6	
Gesunde	83	1	1.2	10	12.0	72	86.8
BCG-Geimpfte	67	3	4.5	17	25.4	47	70.1
Zusammen	385						

Tabelle 16.

Auf den 24- und 48-Std.-Werten fassende Ergebnisse der Mantoux-Wiederholungsreaktion zusammengekommen, wobei das Resultat als gleich galt, wenn die für beide Unterarme ausgerechneten Mittelwerte zweier winkelter Rötungsdurchmesser einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung den 48-Std.-Werten nach						Gleichbleiben den 48-Std.-Werten nach						Steigerung den 48-Std.-Werten nach					
		Abschwächung den 24-Std.-Werten nach		Gleichbleiben den 24-Std.-Werten nach		Steigerung den 24-Std.-Werten nach		Abschwäch. den 24-Std.-Werten nach		Gleichbleiben den 24-Std.-Werten nach		Steigerung den 24-Std.-Werten nach		Abschwäch. den 24-Std.-Werten nach		Gleichbleiben den 24-Std.-Werten nach		Steigerung den 24-Std.-Werten nach	
		Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%
Tub.pulm. I	46	2	4	3	7	7	15	3	7	7	15	8	17	—	—	5	11	11	24
Tub.pulm. II	50	2	4	6	12	4	16	2	4	6	12	9	18	3	6	1	2	13	26
Tub.pulm. III	59	5	8	10	17	1	7	1	2	14	24	6	10	2	3	6	10	11	19
Tub.pulm. zusammen	155	9	5.8	19	12.3	19	12.3	6	3.9	27	17.4	23	14.8	5	3.2	12	7.7	35	22.6
Tub. inactiva	12	—	—	—	—	—	—	—	—	—	—	2	17	1	—	2	—	7	—
Pleuritis exsudativa	48	—	—	2	4	—	—	—	—	4	8	8	17	—	—	8	17	26	54
Sonstige Tuberkulose	2	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Suspekte Tuberkulose	9	1	—	1	—	—	—	1	—	—	—	2	—	—	—	1	—	3	—
Andere Krankheiten	9	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	6	—
Gesunde	83	2	2.4	—	—	—	4.8	—	—	2	2.4	6	7.2	—	—	3	3.6	66	79.6
BCG-Geimpfte ...	67	3	4.5	8	12	22	32.8	—	—	1	1.5	10	14.9	—	—	1	1.5	22	32.8
	385																		

In bezug auf die obigen Resultate sei erwähnt, dass 49.7 % der Lungentuberkulosefälle und 91.6 % der Gesunden eine gesteigerte Sensibilität zeigten. Die Steigerung ist also in beiden Gruppen häufiger als nach 48 Stunden (Tab. 4), aber während nur bei 8.4 % der Gesunden Hemmung und einmal Gleichbleiben vorkam, war die entsprechende Prozentziffer in den Lungentuberkulosefällen etwa sechsmal so hoch, d. h. 50.3 %. Somit haben auch die 24-Std.-Werte einen bedeutenden diagnostischen Wert für die Frage von etwaiger Hemmung oder Gleichheit.

Wenn man bei der erneuten Mantoux-Prüfung nach 24 Stunden das Resultat als Gleichbleiben betrachtete, wenn der Summenunterschied der für beide Unterarme ausgerechneten Mittelwerte zweier winkelrechter Rötungsdurchmesser kleiner war als 10 bzw. 30 %, kommt man zu den in Tabelle 14 und 15 mitgeteilten Resultaten.

Wie aus den Tabellen 14 und 15 hervorgeht, sind auch nach 24 Stunden Gleichbleiben und geringe Abschwächung sehr typisch für die Lungentuberkulose, und die Steigerungen, die in den Lungentuberkulosefällen auftreten, sind zum grossen Teil von geringer Stärke.

Ebenso wie mit den 48-Std.-Werten verhält es sich auch mit den 24-stündigen in bezug auf Abschwächung und Gleichbleiben als Symptom der Lungentuberkulose: die Berechnungsweise gibt nämlich das schärfste Resultat, wo nur das als Gleichbleiben gilt, wenn der Summenunterschied der für beide Unterarme ausgerechneten Mittelwerte zweier Rötungsdurchmesser weniger als 10 % ausmacht. Betrachtet man wiederum die Steigerung der Sensibilität als ein Gesundheitszeichen, so kommt es am deutlichsten zum Vorschein, wenn die besagte Prozentziffer niedriger ist als 30. Der beste Mittelweg ist also, die Grenze bei 20 zu setzen.

Um einen Hinweis dafür zu erhalten, was für etwaige Vorteile die Benutzung der 24- und 48-stündigen Werte zusammen bei der Beurteilung der Resultate der erneuten Mantoux-Reaktion darbieten, wenn der Summenunterschied der für beide Unterarme ausgerechneten Durchmesserwerte kleiner ist als 20 %, sind in Tabelle 16 die auf 24- und 48-stündigen Werten fussenden Resultate wiedergegeben.

Wie aus der obigen Zusammenstellung hervorgeht, zeigten 2

Tabelle 17.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	21	6	29	8	38	7	33
Tubl. pulm. II	33	13	39	14	43	6	18
Tub. pulm. III	46	13	28	21	46	12	26
Tub. pulm. zusammen ..	100	32	32.0	43	43.0	25	25.0
Tub. inactiva	13	1		4		8	
Pleuritis exsudativa	15	5	33	4	27	6	40
Sonstige Tuberkulose	6	3		2		1	
Suspekte Tuberkulose	5	1		—		4	
Andere Krankheiten	13	1		2		10	
Gesunde	195	26	13.3	63	32.3	106	54.4
BCG-Geimpfte	49	13	26.5	23	47.0	13	26.5
Zusammen	396						

Gesunde, deren Sensibilität nach 24 Stunden abgeschwächt war, auch nach 48 Stunden dieselbe Erscheinung. In den Lungentuberkulosefällen fand man nach 24 Stunden bei 12.9 %, und bei einem Viertel (3.2 %) derselben nach 48 Stunden Abschwächung, ein Ergebnis, welches der vorliegenden Untersuchung nach ebenfalls auf aktive Tuberkulose hindeutet.

Somit kann also die Verwendung der 48- und 24-stündigen Werte zusammen einen kleinen diagnostischen Hilfsbeitrag zum erneuten Mantoux liefern.

Die bei der Pirquet-Wiederholungsprüfung auf Grund des 24-stündigen Wertunterschieds ausgerechneten Resultate sind in Tabelle 17 zusammengestellt.

Vergleicht man die Resultate der Lungentuberkulose mit denjenigen der Gesunden, so ergibt es sich, dass sie besser sind als die nach den 48-Std.-Werten (Tab. 7) ausgerechneten. Nur 25 % der Lungenkranken zeigten Sensibilitätssteigerung, während von 195

Tabelle 18.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 10 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.	21	10	48	2	9	9	43
Tub. pulm. II	33	16	49	6	18	11	33
Tub. pulm. III	46	15	33	10	22	21	45
Tub. pulm. zusammen ..	100	41	41	18	18	41	41
Tub. inactiva	13	2		3		8	
Pleuritis exsudativa	15	5		3		7	
Sonstige Tuberkulose	6	3		2		1	
Suspekte Tuberkulose	5	1		—		4	
Andere Krankheiten	13	1		1		11	
Gesunde	195	42	21.5	38	19.5	115	59.0
BCG-Geimpfte	49	21	43	10	20	18	37
Zusammen	396						

Gesunden 106, d. h. 54.4 %, bei der erneuten Prüfung Steigerung aufwiesen.

Der erneute Pirquet legt also wenigstens in den hier untersuchten Fällen dar, dass der Unterschied der 24-stündigen Werte eine grössere Rolle spielt als derjenige der 48-stündigen.

Gruppiert man nun die auf 24 Stunden bezüglichen Ergebnisse des erneuten Pirquet in der Weise, dass ein unter 10 % liegender Summenunterschied zwischen den Mittelwerten zweier winkelrechter Rötungsdurchmesser an beiden Unterarmen die gleiche Sensibilität bezeichnet, so kommt man zu den in Tabelle 18 mitgeteilten Resultaten.

Ein Vergleich zwischen diesen und den in Tabelle 17 stehenden Resultaten ergibt, dass das Steigerungsprozent in den Lungentuberkulosefällen von 25 auf 41 und bei den Gesunden von 54.4 auf 59 zugenommen hat. Also ist die Prozentziffer der erhöhten Sensibilität bei den Lungenkranken mehr gestiegen als bei den Gesunden, was also denselben Sachverhalt darlegt, den wir auch im erneu-

Tabelle 19.

Ergebnisse der Pirquet-Wiederholungsreaktion, wenn das Resultat nur dann als gleich galt, falls die nach 24 Std. für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 30 % erreichte.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I.....	21	2	9	15	72	4	19
Tub. pulm. II	33	4	12	26	79	3	9
Tub. pulm. III	46	9	19	27	59	10	22
Tub. pulm. zusammen ..	100	15	15	68	68	17	17
Tub. inactiva	13	1		4		8	
Pleuritis exsudativa	15	3		6		6	
Sonstige Tuberkulose	6	1		4		1	
Suspekte Tuberkulose	5	—		2		3	
Andere Krankheiten	13	—		4		9	
Gesunde	195	14	7.2	89	45.6	92	47.2
BCG-Geimpfte	49	6	12	34	70	9	18
Zusammen	396						

ten Mantoux fanden, nämlich, dass bei Lungentuberkulose relativ oft leichtere Steigerungen vorkommen. Gleichzeitig ergibt es sich, dass man ein Resultat am besten als gleich bezeichnen kann, wenn der besagte Unterschied kleiner ist als 20 %.

Zieht man die Grenze des Gleichbleibens bei 30 %, so kommt man zu den in Tabelle 19 eingetragenen Resultaten.

Nach dieser Berechnungsweise ist bei Lungentuberkulose nur in 17 % der Fälle, aber bei 47.2 % der Gesunden Steigerung wahrzunehmen. Auch diese Ergebnisse weisen ihrerseits darauf hin, dass bei Lungenkranken verhältnismässig oft schwächere Steigerungen auftreten als bei Gesunden. Das Verhältnis der Steigerungsprozente zwischen Lungenkranken und Gesunden ist in der That vorteilhafter, als wenn die Bestimmung des Gleichbleibens sich nach dem hinter 20 % zurückbleibenden Unterschied richtet (Tab. 17), aber in Analogie mit den Berechnungsgründen bei der Mantoux-Wiederholungsreaktion richten sich die Ergebnisse bei der Pirquet-

Tabelle 20.

Auf 24-Std.-Werten fussende Ergebnisse der Pirquet-Wiederholungsreaktion bei Verwendung verschieden langer Zwischenzeiten, wenn die für beide Unterarme ausgerechneten Mittelwerte von zwei winkelrechten Rötungsdurchmessern einen Summenunterschied zwischen der ersten und der erneuten Prüfung ergaben, der nicht 20 % erreichte.

Inter- vall	Art der Fälle	Anzahl der Fälle	Abschwä- chung		Gleich- bleiben		Steigerung	
			der Sensibilität					
			An- zahl	%	An- zahl	%	An- zahl	%
1 Monat	Gesunde	21	1	5	4	19	16	76
14 Tage	Tub. pulm. I	3	3		—		—	
	Tub. pulm. II	5	3		2		—	
	Tub. pulm. III	14	6		6		2	
	Tub. pulm. zusammen ..	22	12	55	8	36	2	9
	Tub. inactiva	1	—		—		1	
	Sonstige Tuberkulose	2	1		1		—	
	Suspekte Tuberkulose ..	3	—		—		3	
	Andere Krankheiten	1	—		—		1	
	Gesunde	32	5	15	6	19	21	66
	Zusammen	61						
5 Tage	Tub. pulm. I	18	3		8		7	
	Tub. pulm. II	28	10		12		6	
	Tub. pulm. III	32	7		15		10	
	Tub. pulm. zusammen ..	78	20	26	35	45	23	29
	Tub. inactiva	12	1		4		7	
	Pleuritis exsudativa	15	5		4		6	
	Sonstige Tuberkulose	4	2		1		1	
	Suspekte Tuberkulose	2	—		1		1	
	Andere Krankheiten	12	1		2		9	
	Gesunde	142	20	14	53	37	69	49
BCG-Geimpfte	45	13	26.1	23	47.0	13	26.5	
Zusammen	314							

Wiederholungsreaktion im Folgenden danach, dass die Grenze des Gleichbleibens bei einem Unterschied von weniger als 20% gezogen wird.

Wenn man in der Pirquet-Wiederholungsreaktion die Resultate auf Grund des Unterschieds der 24-Std.-Werte, nach Intervallen von 1 Monat, 14 und 5 Tagen in Gruppen einteilt, ergibt sich Tabelle 20.

Wie ersichtlich, trat bei 21 Gesunden, bei denen die erneute Pirquet-Probe nach einem Monat stattfand, 16mal, d. h. in 76 %, Steigerung der Sensibilität auf. Betrug die Zwischenzeit 14 Tage, zeigten 2, d. h. 9 %, Steigerung; dasselbe war bei 21, d. h. 66 %, von 32 Gesunden der Fall. In 78, nach 5 Tagen untersuchten Lungentuberkulosefällen kam 23mal, d. h. in 29 %, Steigerung vor, während 69 von 142 Gesunden, d. h. 49 %, bei erneuter Prüfung gesteigerte Sensibilität zeigten.

Die Resultate weisen darauf hin, dass die Pirquet-Wiederholungsreaktion noch nach einem Monat Steigerung bei Gesunden aufweist. Dabei zeigte es sich recht deutlich, dass die gewählte fünftägige Zwischenzeit zu kurz war, aber dass ein 14-tägiges Intervall zu besseren Resultaten geführt hätte.

Zusammenfassend kann von der obenbeschriebenen Methodik gesagt werden, dass es im erneuten Mantoux die brauchbarste Methode ist, das Ergebnis als Gleichbleiben zu bezeichnen, wenn man für die Rötungen, die durch Impfung an zwei verschiedenen Stellen hervorgerufen sind, die Mittelwerte zweier winkelrechter Durchmesser bestimmt und ihren Summenunterschied bei der ersten und der erneuten Reaktion kleiner findet als 20 %. Dabei hat es sich auch gezeigt, dass schon eine geringe Abschwächung im erneuten Mantoux sehr typisch ist für die Lungentuberkulose und dass die in Lungentuberkulosefällen vorkommenden Steigerungen zu einem relativ grossen Teil von geringer Stärke sind.

Im erneuten Mantoux ist es vorteilhafter, den von den 48-Std.-Werten dargelegten Unterschied als Beurteilungsgrundlage zu benutzen, während im erneuten Pirquet die Unterschiede der 24-Std.-Werte entschieden bessere Resultate geben. Bei Verwendung der letztgenannten Werte erhält man im erneuten Pirquet brauchbarere Resultate, wenn das Gleichbleiben der Sensibilität sich nach einem Unterschied unter 20 % richtet. Auch die erneute Pirquet-Prüfung hat dargelegt, dass die bei Lungentuberkulose auftretende Steigerung meistens nur eine geringfügige ist.

Bei meinen Versuchen, zu erforschen, welches Intervall in der Tuberkulinwiederholungsprobe das brauchbarste wäre, fand ich, dass 14 Tage im erneuten Mantoux vielleicht etwas bessere Resultate liefern als 5 Tage, aber dass im erneuten Pirquet 14 Tage ganz deutlich eine günstigere Zwischenzeit bilden, als 5 Tage

Klinische Ergebnisse.

In der folgenden Darstellung des Verlaufs der nach Krankheitsgruppen geordnet stattfindenden Tuberkulinwiederholungsprüfung wird das Resultat als Gleichbleiben der Sensibilität betrachtet, wenn die für beide Unterarme ausgerechneten Mittelwerte zweier winkelrechter Rötungsdurchmesser einen geringeren Summenunterschied zwischen der ersten und der erneuten Reaktion ergeben, als 20 %. Im erneuten Mantoux kamen die nach 48 Stunden, im erneuten Pirquet die nach 24 Stunden gewonnenen Werte zur Anwendung. Verschieden lange Intervalle wurden sowohl bei der Mantoux- wie bei der Pirquet-Wiederholungsprüfung verwendet, doch werden die Resultate trotzdem im Folgenden zusammen vorgelegt, obwohl die Benutzung verschiedener Zwischenräume in gewissem Grade voneinander abweichende Resultate bewirkte.

Die Übersicht der in den einzelnen Krankheitsgruppen gewonnenen Resultate findet sich für Mantoux in Tabelle 4 und für Pirquet in Tabelle 17.

Lungentuberkulose. Es wurden im ganzen 255 Fälle von aktiver Lungentuberkulose untersucht, davon 155 mit der Mantoux- und 100 mit der Pirquet-Wiederholungsreaktion.

Im erneuten Mantoux zeigten von 155 Lungenkranken 47, d. h. 30.3 %, Abschwächung, 56, d. h. 36.1 %, gleiches Resultat und 52, d. h. 33.6 %, Steigerung. Nach dem Krankheitsstadium (Umfang der Veränderungen) eingeteilt, war die Sensibilität in 46 Fällen I. Grades 12mal, d. h. in 26 %, abgeschwächt, 18mal, d. h. in 39 %, unverändert und 16mal, d. h. in 35 %, gesteigert. Von 50 Fällen II. Grades zeigten 16, d. h. 32 %, Abschwächung, 17, d. h. 34 %, gleiches Resultat und ebenfalls 17, d. h. 34 %, Steigerung. Fälle III. Grades gab es 59, davon 19, d. h. 32 %, mit Abschwächung, 21, d. h. 36 %, mit gleichem Resultat und 19, d. h. 32 %, mit Steigerung.

Wie ersichtlich, sind die Prozentziffern in verschiedenen Krankheitsstadien ungefähr die gleichen, wenigstens scheint die erneute Mantoux-Reaktion weder im Anfangsstadium der Krankheit noch in weiter fortgeschrittenen Fällen besonders typisch zu sein; vergleicht man aber die Resultate der Tabellén 3, 4 und 5 so miteinander, dass man die Prozentziffern der Steigerungsfälle in den einzelnen Stadien der Lungentuberkulose nacheinander reiht, je nachdem, ob die beobachtete Steigerung schwach (10 %), mässig (20 %) oder stärker (30 %) war, so ergibt sich die Zusammenstellung 4.

Zusammenstellung 4.

Mit Berücksichtigung

	schwacher (10 %) Steigerung	mässiger (20 %) Steigerung	stärkerer (30 %) Steigerung
Tub. pulm. I.	46 %	35 %	22 %
Tub. pulm. II.	48 %	34 %	24 %
Tub. pulm. III.	39 %	32 %	29 %

Man findet hier, dass die Prozentziffern der Steigerung im I. und II. Stadium der Lungentuberkulose schärfer abfallen als im III. Stadium, wenn ausser den schwachen Steigerungen auch die mässigen (20%) und dann die stärkeren (30%) berücksichtigt werden. Dieser Sachverhalt seinerseits legt wiederum dar, dass schwächere Steigerungen häufiger im I. und II. Stadium der Lungentuberkulose als im III. Stadium derselben vorkommen. In den einzelnen Stadien der Lungentuberkulose zeigt die wiederholte Mantoux-Reaktion ungefähr dasselbe Verhalten, indem etwa ein Drittel der Fälle gesteigerte Sensibilität aufweist, aber schwächere Steigerungen im I. und II. Stadium der Krankheit häufiger als im III. Stadium auftreten.

Eine Betrachtung der Resultate der Pirquet-Wiederholungsreaktion ergibt, dass von 100 Lungentuberkulosefällen 32 % Abschwächung, 43 % Gleichbleiben und 25 % Steigerung aufwiesen. Ebenso wie oben in drei Krankheitsstadien klassifiziert, fand man in 21 Fällen von Lungentuberkulose I. Grades sechsmal, d. h. in 29 %, eine abgeschwächte, achtmal, d. h. in 38 %, eine unveränderte und siebenmal, d. h. in 33 %, eine gesteigerte Sensibilität. Die Fälle II. Grades waren 33 an der Zahl, davon 13, d. h. 39 %, mit Abschwächung, 14, d. h. 43 %, mit gleichem Resultat

Tabelle 21.

Vergleich zwischen den auf 48-Std.-Werten fussenden Ergebnissen der Mantoux-Wiederholungsreaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	29	7		16		6	
Tub. pulm. II ohne Pneumoth. art.	25	9		8		8	
Tub.pulm. III ohne Pneumoth. art	48	16		17		15	
Zusammen	102	32	31	41	40	29	29
Tub. pulm. I mit Pneumoth. art.	17	5		2		10	
Tub. pulm. II mit Pneumoth. art.	25	7		9		9	
Tub. pulm. III mit Pneumoth. art.	11	3		4		4	
Zusammen	53	15	28	15	28	23	44

und 6, d.h. 18 %, mit Steigerung. Von 46 Fällen III. Grades zeigten 13, d.h. 28 %, Abschwächung, 21, d.h. 46 %, Gleichbleiben und 12, d.h. 26 %, Steigerung. Unter den Fällen II. Grades war die Menge der Steigerungen am geringsten, nämlich 18 %, doch ist die Anzahl der Fälle in den einzelnen Gruppen überhaupt so klein, dass sie keine besonderen Schlüsse erlauben dürften.

Beim Studium der etwaigen Wirkung der Kollapsbehandlung auf die Tuberkulinwiederholungsreaktion wurde nur der Pneumothorax berücksichtigt, denn die sonstigen Kollapsbehandlungsfälle meines Materials bestanden nur aus ganz wenigen, vor verschieden langen Zeiten ausgeführten Thorakoplastiken oder Phrenikoexhairen. Die mit erneutem Mantoux untersuchten Lungentuberkulosefälle, die während der Untersuchung Pneumothoraxbehandlung erhielten, waren 53 an der Zahl. Der Vergleich dieser Fälle mit den ohne Pneumothorax behandelten ist in Tabelle 21 wiedergegeben.

23 der untersuchten 53 Pneumothoraxfälle, d.h. 44 %, zeigten bei erneuter Mantoux-Prüfung Steigerung der Sensibilität, während bei 102 anderen Fällen nur 29 mal, d.h. in 29 %, eine solche nachzuweisen war.

Tabelle 22.

Vergleich zwischen den auf 48-Std.-Werten fussenden Ergebnissen der nach 5 Tagen wiederholten Mantoux-Reaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	21	4		12		5	
Tub. pulm. II ohne Pneumoth. art.	24	8		8		8	
Tub. pulm. III ohne Pneumoth. art.	34	8		14		12	
Zusammen	79	20	25	34	43	25	32
Tub. pulm. I mit Pneumoth. art.	15	4		2		9	
Tub. pulm. II mit Pneumoth. art.	18	4		5		9	
Tub. pulm. III mit Pneumoth. art.	10	2		4		4	
Zusammen	43	10	23	11	26	22	51

Um zu erforschen, welchen Anteil die Benutzung verschieden langer Intervalle bei der Mantoux-Wiederholungsreaktion eventuell an dem Unterschied zwischen den erwähnten Prozentzahlen besitzt, wurden in Tabelle 22 die nach 5-tägigem und in Tabelle 23 die nach 14-tägigem Intervall untersuchten Fälle, in gleicher Weise verteilt, eingetragen.

Die Zahl der nach 5-tägigem Intervall untersuchten Pneumothoraxfälle betrug 43, von denen 22, d.h. 51 %, eine gesteigerte Sensibilität aufwiesen. Die Lungentuberkulosefälle, die während der Untersuchungszeit keine Pneumothoraxbehandlung erhielten und nach 5-tägiger Zwischenzeit geprüft wurden, waren 79 an der Zahl, davon 25, d.h. 32 %, mit Steigerung. Von den 10 nach 14-tägiger Zwischenzeit untersuchten Pneumothoraxfällen zeigte 1, d.h. 10 %, gesteigerte Sensibilität. Fälle ohne Pneumothorax, bei denen ein Intervall von 14 Tagen zur Verwendung kam, gab es 23, davon 4, d.h. 17 %, mit Steigerung. Aus den Resultaten ist also zu entnehmen, dass die nach 14-tägigem Intervall untersuchten Fälle ausgleichend auf den im ganzen Material zum Vorschein kommenden Unterschied zwischen den Fällen mit und

Tabelle 23.

Vergleich zwischen den auf 48-Std.-Werten fussenden Ergebnissen der nach 14 Tagen wiederholten Mantoux-Reaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	8	3		4		1	
Tub. pulm. II ohne Pneumoth. art.	1	1		—		—	
Tub. pulm. III ohne Pneumoth. art	14	8		3		3	
Zusammen	23	12	52	7	31	4	17
Tub. pulm. I mit Pneumoth. art.	2	1		—		1	
Tub. pulm. II mit Pneumoth. art.	7	3		4		—	
Tub. pulm. III mit Pneumoth. art	1	1		—		—	
Zusammen	10	5	50	4	40	1	10

ohne Pneumothorax bei der Mantoux-Wiederholungsprüfung wirken.

Die gefundenen Resultate weisen darauf hin, dass im erneuten Mantoux wenigstens bei Verwendung 5-tägiger Intervalle eine Sensibilitätssteigerung häufiger in Pneumothoraxfällen als bei anderen Lungentuberkulösen vorkommt. Somit nähme die Pneumothorax-Gruppe eine Mittelstellung zwischen den Lungentuberkulösen und den Gesunden ein.

Gruppiert man die mit erneuter Pirquet-Reaktion untersuchten Fälle von aktiver Lungentuberkulose je nachdem, ob eine Pneumothoraxbehandlung vorkam oder nicht, so findet man für die Schwankungen der 24-Std.-Werte die in Tabelle 24 mitgeteilten Ergebnisse.

Die Zahl der mit erneutem Pirquet untersuchten Lungenkranken ohne Pneumothorax betrug 84, davon 23, d.h. 27 %, mit gesteigerter Sensibilität. An Pneumothoraxfällen gab es nur 16, von welchen 2, d.h. 12.5 %, Steigerung zeigten. Der erneute Pirquet lieferte somit ein entgegengesetztes Resultat als der wiederholte Mantoux, aber die Anzahl der Pneumothoraxfälle ist dermassen gering (16), dass sie wohl keine Schlüsse erlaubt.

Tabelle 24.

Vergleich zwischen den auf 24-Std.-Werten fussenden Ergebnissen der Pirquet-Wiederholungsreaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	18	4		7		7	
Tub. pulm. II ohne Pneumoth. art.	24	9		10		5	
Tub. pulm. III ohne Pneumoth. art.	42	13		18		11	
Zusammen	84	26	31	35	42	23	27
Tub. pulm. I mit Pneumoth. art.	3	2		1		—	
Tub. pulm. II mit Pneumoth. art.	9	4		4		1	
Tub. pulm. III mit Pneumoth. art.	4	—		3		1	
Zusammen	16	6	37.5	8	50.0	2	12.5

In den Tabellen 25 und 26 sind die Fälle danach eingeteilt, ob die Zwischenzeit 5 oder 14 Tage ausmachte.

Die Fälle ohne Pneumothorax, die nach 5 Tagen untersucht wurden, waren 66 an der Zahl und 21 derselben, d.h. 32 %, zeigten gesteigerte Sensibilität, während es 12 Pneumothoraxfälle gab, und zwar 2, d.h. 17 %, mit Steigerung. Nach einem Intervall von 14 Tagen wurden 18 Fälle ohne Pneumothorax untersucht und 2 derselben, d.h. 11 %, wiesen Steigerung auf; diesen entsprachen 4 Fälle mit Pneumothorax, sämtliche ohne Steigerung.

Bei der erneuten Pirquet-Prüfung, wo die Schwankungen der 24-Std.-Werte als Abschätzungsgrundlage dienten, zeigten die Pneumothoraxfälle im Vergleich zur erneuten Mantoux-Prüfung ein entgegengesetztes Verhalten, indem Steigerung bei den Lungenkranken ohne Pneumothorax häufiger auftrat; aber auch hier ist die Zahl der untersuchten Pneumothoraxfälle wohl zu gering (16), um Schlussfolgerungen zu erlauben.

Wenn die mit der Mantoux-Wiederholungsreaktion untersuchten, an aktiver Lungentuberkulose Leidenden in zwei Gruppen eingeteilt werden, je nachdem, ob während der Untersuchungszeit Fieber vorgekommen war — abgesehen von einer etwaigen Tem-

Tabelle 25.

Vergleich zwischen den auf 24-Std.-Werten fussenden Ergebnissen der nach 5 Tagen wiederholten Pirquet-Reaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	17	3		7		7	
Tub. pulm. II ohne Pneumoth. art.	20	7		8		5	
Tub. pulm. III ohne Pneumoth. art.	29	7		13		9	
Zusammen	66	17	26	28	42	21	32
Tub. pulm. I mit Pneumoth. art.	1	—		1		—	
Tub. pulm. II mit Pneumoth. art.	8	3		4		1	
Tub. pulm. III mit Pneumoth. art.	3	—		2		1	
Zusammen	12	3	25	7	58	2	17

Tabelle 26.

Vergleich zwischen den auf 24-Std.-Werten fussenden Ergebnissen der nach 14 Tagen wiederholten Pirquet-Reaktion in betreff der ohne und mit Pneumothorax behandelten Fälle von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
		der Sensibilität					
		Anzahl	%	Anzahl	%	Anzahl	%
Tub. pulm. I ohne Pneumoth. art.	1	1		—		—	
Tub. pulm. II ohne Pneumoth. art.	4	2		2		—	
Tub. pulm. III ohne Pneumoth. art.	13	6		5		2	
Zusammen	18	9	50	7	39	2	11
Tub. pulm. I mit Pneumoth. art.	2	2		—		—	
Tub. pulm. II mit Pneumoth. art.	1	1		—		—	
Tub. pulm. III mit Pneumoth. art.	1	—		1		—	
Zusammen	4	3	75	1	25	—	—

Tabelle 27.

Auf 48-Std.-Werten fussende Ergebnisse der Mantoux-Wiederholungsreaktion in fieberhaften und fieberfreien Fällen von Lungentuberkulose.

Art der Fälle		Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
			der Sensibilität					
			Anzahl	%	Anzahl	%	Anzahl	%
Fieberhaft {	Tub. pulm. I	9	3		5		1	
	Tub. pulm. II	11	6		4		1	
	Tub. pulm. III	14	6		6		2	
Zusammen		34	15	44	15	44	4	12
Fieberfrei {	Tub. pulm. I	37	9		13		15	
	Tub. pulm. II	39	10		13		16	
	Tub. pulm. III	45	13		15		17	
Zusammen		121	32	26	41	34	48	40

peraturerhöhung infolge der Tuberkulinprobe selbst —, oder ob die Untersuchungszeit fieberlos verlief, und wenn noch dazu die verschiedenen Krankheitsstadien berücksichtigt werden, so ergeben sich die in Tabelle 27 zusammengestellten Resultate.

Unter den Resultaten sei hervorgehoben, dass von den 34 fiebernden Fällen 4, d.h. 12 %, Steigerung aufwiesen, während dies bei 48, d.h. 40 %, der 121 fieberfreien Fälle vorkam. Die Ergebnisse der erneuten Mantoux-Prüfung gehen also dahin, dass man Abschwächung oder Gleichbleiben in fiebernden Lungentuberkulosefällen häufiger als in fieberfreien feststellen kann.

Eine in zuletzt beschriebener Weise nach den Unterschieden der 24-Std.-Werte im erneuten Pirquet vollzogene Gruppeneinteilung führt zu den in Tabelle 28 vorgelegten Resultaten.

Wie ersichtlich, trat bei 8, d.h. 18 % der 46 fiebernden Fälle Steigerung auf, während dies bei 17, d.h. 32 % der 54 fieberfreien Fälle vorkam. Somit stimmen die Resultate der erneuten Pirquet-Reaktion mit denjenigen der entsprechenden Mantoux-Prüfung überein; demnach findet man Abschwächung oder Gleichbleiben in fiebernden Fällen von Lungentuberkulose häufiger als in fieberfreien.

Tabelle 28.

Auf 24-Std.-Werten fussende Ergebnisse der Pirquet-Wiederholungsreaktion in fieberhaften und fieberfreien Fällen von Lungentuberkulose.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung		
		der Sensibilität						
		Anzahl	%	Anzahl	%	Anzahl	%	
Fieberhaft {	Tub. pulm. I	6	1		4		1	
	Tub. pulm. II	12	7		5		—	
	Tub. pulm. III	28	11		10		7	
Zusammen		46	19	41	19	41	8	18
Fieberfrei {	Tub. pulm. I	15	5		4		6	
	Tub. pulm. II	21	6		9		6	
	Tub. pulm. III	18	2		11		5	
Zusammen		54	13	24	24	44	17	32

Als Schlussfolgerung aus der obigen Darstellung kann gesagt werden, dass bei der Tuberkulinwiederholungsreaktion eine Abschwächung oder ein Gleichbleiben der Sensibilität in fiebernden Fällen von Lungentuberkulose deutlich eine gewöhnlichere Erscheinung ist, als in fieberfreien.

Vergleicht man die Ergebnisse des erneuten Mantoux damit, ob in dem betreffenden Fall zur Zeit der Tuberkulinprüfungen bei der üblichen Sputumfärbung Tuberkelbazillen zum Vorschein gekommen sind oder nicht, so findet man die in Tabelle 29 dargestellten Resultate.

In 82 Fällen wurden zur Zeit der wiederholten Mantoux-Untersuchung mit Hilfe der üblichen Färbungsmethode keine Tuberkelbazillen gefunden, und in 29, d.h. 35 % von jenen Fällen war Steigerung bemerkbar. Unter denjenigen 73 Fällen, wo die gewöhnliche Sputumuntersuchung zur Zeit der Mantoux-Reaktion keine Tuberkelbazillen aufdeckte, gab es 23, d.h. 31 %, mit gesteigerter Sensibilität. Der Unterschied zwischen den gefundenen Prozentziffern ist jedoch zu gering, um Schlussätze zu erlauben; zudem ist in unseren Krankenhäusern das Finden nach Tuberkelbazillen nicht vielseitig genug, da es nicht einmal möglich gewesen ist, eine allgemeinere Bazillenzüchtung einzuführen.

Tabelle 29.

Auf 48-Std.-Werten fassende Ergebnisse der Mantoux-Wiederholungsreaktion in Lungentuberkulosefällen, die in zwei Klassen eingeteilt sind, je nachdem, ob zur Zeit der Tuberkulinuntersuchungen Tuberkelbazillen im Auswurf vorkamen oder nicht.

Art der Fälle		Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
			der Sensibilität					
			Anzahl	%	Anzahl	%	Anzahl	%
Keine Tuberkelbazillen im Auswurf nachgewiesen	Tub. pulm. I	35	10		14		11	
	Tub. pulm. II	28	10		8		10	
	Tub. pulm. III	19	6		5		8	
Zusammen		82	26	32	27	33	29	35
Tuberkelbazillen im Auswurf nachgewiesen	Tub. pulm. I	11	2		4		5	
	Tub. pulm. II	22	6		9		7	
	Tub. pulm. III	40	13		16		11	
Zusammen		73	21	29	29	40	23	31

Tabelle 30.

Auf 24-Std.-Werten fassende Ergebnisse der Pirquet-Wiederholungsreaktion in Lungentuberkulosefällen, die in zwei Klassen eingeteilt sind, je nachdem, ob zur Zeit der Tuberkulinuntersuchungen Tuberkelbazillen im Auswurf vorkamen oder nicht.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung		
		der Sensibilität						
		Anzahl	%	Anzahl	%	Anzahl	%	
Keine Tuberkelbazillen im Auswurf nachgewiesen	Tub. pulm. I	17	5		6		6	
	Tub. pulm. II	20	7		8		5	
	Tub. pulm. III	2	—		2		—	
Zusammen		39	12	31	16	41	11	28
Tuberkelbazillen im Auswurf nachgewiesen	Tub. pulm. I	4	1		2		1	
	Tub. pulm. II	13	6		6		1	
	Tub. pulm. III	44	13		19		12	
Zusammen		61	20	33	27	44	14	23

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Die entsprechenden Resultate des erneuten Pirquet, auf Grund der Schwankungen der 24-Std.-Werte ausgerechnet, finden sich in Tabelle 30.

Es gab 39 Fälle, wo die gewöhnliche Färbemethode nicht zur Zeit der wiederholten Pirquet-Reaktion Tuberkelbazillen zum Vorschein brachte; hier wurde elfmal, d.h. in 28 % der Fälle, Steigerung gefunden. Mit der üblichen Färbemethode wurden zur Zeit der Pirquet-Untersuchungen Tuberkelbazillen in 61 Fällen nachgewiesen, und 14, d.h. 23 % derselben liessen Steigerung erkennen. *Der Unterschied zwischen den bazillenpositiven und den bazillen-negativen Fällen ist also ebenso gross und von derselben Art bei den mit Mantoux und mit Pirquet Untersuchten, aber auch ebenso unbedeutend, so dass es nicht möglich sein dürfte, irgendwelche Schlüsse daraus zu ziehen.*

Um die Ergebnisse der Tuberkulinwiederholungsreaktion mit den Resultaten der Senkungsreaktion der roten Blutkörperchen (nach Westergren) vergleichen zu können, wurden die Fälle in drei Gruppen eingeteilt, je nachdem, ob nach Westergrens Methode der Wert während der ersten Stunde 0—8 mm, 9—30 mm oder mehr als 30 mm ausmachte. Die Ergebnisse der Tuberkulinwiederholungsreaktion finden sich, in dieser Weise eingeteilt, in Tabelle 31.

Die mit dem erneuten Mantoux untersuchten, zur ersten Gruppe gehörenden Fälle waren 41 an der Zahl und zeigten somit nach Westergren während der ersten Stunde den Wert 0—8 mm; in 19, d.h. 46 % derselben, trat Steigerung auf. Falls Westergren zur Zeit der Untersuchung den Wert 9—30 mm lieferte, so fand man im erneuten Mantoux unter 61 Fällen 22, d.h. 36 %, mit Steigerung, während bei einem Wert von mehr als 30 mm in 53 Fällen nur elfmal, d.h. in 21 %, Steigerung auftrat. Die Resultate der Mantoux-Wiederholungsreaktion weisen also darauf hin, dass Steigerung in solchen Lungentuberkulosefällen, wo die Senkungsgeschwindigkeit der roten Blutkörperchen (nach Westergren) im Verlauf der ersten Stunde hoch ist, häufiger vorkommt, als in Fällen mit geringer Senkungsgeschwindigkeit.

Im Verlauf der wiederholten Pirquet-Untersuchung gab Westergren innerhalb der ersten Stunde den Wert 0—8 mm in 23 Fällen, und zwar wurde in 9, d.h. 39 % derselben, Steigerung festgestellt. Der entsprechende Wert betrug in 27 Fällen 9—30 mm; hier kam viermal, d.h. in 15 %, ein Steigen der Sensibilität vor. Über 30 mm

Tabelle 32.

Auf 48-Std.-Werten fussende Ergebnisse der Mantoux-Wiederholungsreaktion in Lungentuberkulosefällen, die in zwei Klassen eingeteilt sind, je nachdem, ob röntgenologisch Kavernen nachgewiesen wurden oder nicht.

Art der Fälle	Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung		
		der Sensibilität						
		Anzahl	%	Anzahl	%	Anzahl	%	
Kavernös {	Tub. pulm. I	—	—	—	—	—	—	
	Tub. pulm. II	11	3	5		3		
	Tub. pulm. III	28	9	9		10		
Zusammen		39	12	31	14	36	13	33
Nicht kavernös: {	Tub. pulm. I	46	12	18		16		
	Tub. pulm. II	39	13	12		14		
	Tub. pulm. III	31	10	12		9		
Zusammen		116	35	30	42	36	39	34

hatten nach Westergren während der ersten Stunde 50 Fälle, davon 12, d.h. 24 %, mit Steigerung. Die Tatsache, dass das niedrigste Steigerungsprozent auf die mittlere Gruppe entfiel, kann darauf hinweisen, dass der Zufall im erneuten Pirquet eine grössere Rolle spielt; aber in der dritten Gruppe war jedenfalls die Prozentzahl der Steigerungen niedriger als in der ersten, so dass die Ergebnisse der erneuten Pirquet-Reaktion in dieser Beziehung mit denjenigen von Mantoux übereinstimmen, m.a.W.: die wiederholte Pirquet-Prüfung ergibt öfter Steigerung in solchen Fällen, wo Westergren während der ersten Stunde niedrig ist, als dann, wenn der entsprechende Senkungswert hoch ist.

Als Schlussfolgerung kann gesagt werden, dass die Sensibilität bei wiederholter Tuberkulinprüfung häufiger gesteigert erscheint, wenn die Senkungsreaktion während der ersten Stunde einen niedrigen Wert aufweist, als in Fällen mit hohem (über 30 mm) Wert.

Ich habe ferner die Personen mit aktiver Lungentuberkulose in zwei Gruppen eingeteilt, je nachdem, ob bei der Röntgenuntersuchung eine Kaverne gefunden wurde oder nicht. Die Ergebnisse des erneuten Mantoux in Verbindung mit dieser Einteilung sind in Tabelle 32 zusammengestellt.

Tabelle 33.

Auf 24-Std.-Werten fussende Ergebnisse der Pirquet-Wiederholungsreaktion in Lungentuberkulosefällen, die in zwei Klassen eingeteilt sind, je nachdem, ob röntgenologisch Kavernen nachgewiesen wurden oder nicht.

Art der Fälle		Anzahl der Fälle	Abschwächung		Gleichbleiben		Steigerung	
			der Sensibilität					
			Anzahl	%	Anzahl	%	Anzahl	%
Kavernös	Tub. pulm. I	—	—		—		—	
	Tub. pulm. II	6	2		3		1	
	Tub. pulm. III	21	7		10		4	
	Zusammen	27	9	33	13	48	5	19
Nicht kavernös	Tub. pulm. I	21	6		8		7	
	Tub. pulm. II	27	11		11		5	
	Tub. pulm. III	25	6		11		8	
	Zusammen	73	23	32	30	41	20	27

Die Zahl der erneut mit Mantoux geprüften kavernösen Fälle ist 39, davon 13, d.h. 33 %, mit Steigerung. In 116 Fällen wurden keine Kavernen nachgewiesen, und in 39, d.h. 34 % derselben, war die Sensibilität gesteigert. Auch Abschwächung und Gleichbleiben waren prozentuell ungefähr ebenso in den kavernösen und nicht kavernösen Fällen.

Die Resultate lassen also die Richtung erkennen, dass mit Hilfe der Mantoux-Wiederholungsreaktion kein deutlicher Unterschied zwischen Fällen mit und ohne Kavernen nachgewiesen werden kann.

Die Ergebnisse der erneuten Pirquet-Reaktion in betreff der kavernösen und nicht kavernösen Fälle sind in Tabelle 33 veranschaulicht.

Benutzt man bei der erneuten Pirquet-Prüfung die Unterschiede der 24-Std.-Werte als Beurteilungsgrundlage, so findet man in 27 kavernösen Fällen fünfmal, d.h. in 19 %, und in 73 nicht kavernösen 20mal, d.h. in 27 %, gesteigerte Sensibilität.

Gemäss dem erneuten Pirquet ist also Steigerung in kavernösen Fällen eine seltenere Erscheinung als in nicht kavernösen.

Als Schlussfolgerung und Zusammenfassung der Wiederholungsprüfungen nach

Mantoux und Pirquet bei klinisch aktiver Lungentuberkulose sei erwähnt:

Wird bei der Mantoux-Wiederholungsreaktion der Unterschied der 48-Std.-Werte als Beurteilungsgrund verwendet, so kann festgestellt werden, dass die besagte Reaktion sich in verschiedenen Stadien der Lungentuberkulose ungefähr ebenso verhält, wenn die Fälle je nach der Ausdehnung der Lungenherde in drei Grade eingeteilt werden; doch ist dabei nachzuweisen, dass in den Fällen I. und II. Grades die schwachen Steigerungen zahlreicher sind als in den Fällen III. Grades.

Bei Pneumothorax kommt wenigstens nach 5-tägigem Intervall in der erneuten Mantoux-Reaktion eine Steigerung häufiger vor, als bei Lungenkranken ohne Pneumothorax.

In den zur Untersuchungszeit fieberhaften Fällen von Lungentuberkulose ist Abschwächung oder Gleichbleiben der Sensibilität eine gewöhnlichere Erscheinung, als in den Fällen, die zu der erwähnten Zeit kein Fieber hatten.

Die Resultate der wiederholten Mantoux-Reaktion zeigen keinen grösseren Unterschied, wenn im Verlauf der Tuberkulinuntersuchungen mit Hilfe des üblichen Färbeverfahrens im Auswurf Tuberkelbazillen gefunden wurden oder nicht.

Vergleicht man die beim erneuten Mantoux gewonnenen Resultate mit der Senkungsgeschwindigkeit der roten Blutkörperchen, so findet man häufiger Steigerung in solchen Fällen, wo Westergren innerhalb der ersten Stunde einen niedrigen Wert liefert, als dann, wenn der besagte Wert 30 mm übersteigt.

Hat man die Fälle von Lungentuberkulose in zwei Gruppen eingeteilt, je nachdem, ob eine Kaverne röntgenologisch nachgewiesen worden ist oder nicht, so zeigt es sich, dass in dieser Hinsicht kein bemerkenswerter Unterschied bei der Wiederholungsprüfung nach Mantoux auftritt.

Bei der Wiederholungsprüfung nach Pirquet diene die Schwankung der 24-Std.-Werte als Beurteilungsgrund. Von den untersuchten 100 Lungentuberkulosefällen ergaben 32 % Abschwächung, 43 % Gleichbleiben und 25 % Steigerung der Sensibilität.

In betreff der verschiedenen Grade der Lungentuberkulose war Steigerung im II. Stadium am seltensten.

Bei der erneuten Pirquet-Prüfung war Steigerung eine gewöhn-

lichere Erscheinung in Fällen ohne Pneumothorax, als bei Pneumothoraxpatienten (die Zahl der letzteren betrug nur 16).

Abgeschwächtes oder gleiches Resultat kam auch bei der wiederholten Pirquet-Prüfung in fieberhaften Fällen häufiger vor, als in fieberfreien.

Der Sachverhalt, ob ein Fall zur Zeit der Tuberkulinuntersuchungen mit Hilfe der üblichen Färbemethode nachweisbare Tuberkelbazillen hatte oder nicht, rief auch bei der Wiederholungsprüfung nach Pirquet keinen grösseren Unterschied hervor.

Im erneuten Pirquet zeigte sich Steigerung seltener, wenn Westergren den Wert 9 mm oder mehr lieferte, als in den Fällen, wo der genannte Wert während der ersten Stunde nicht 9 mm erreichte.

Im erneuten Pirquet legten die kavernösen Fälle seltener als diejenigen Fälle, in denen röntgenologisch keine Kaverne gefunden wurde, eine Steigerung der Sensibilität dar.

Inaktive Tuberkulose. Es wurden 25 Fälle mit inaktiver Tuberkulose untersucht. Die Diagnose lautete in diesen Fällen: Tuberculosis pulmonum oder pulmonis inactiva oder inveterata oder auch Tuberculosis lymphoglandularum inactiva oder inveterata. Von diesen Fällen wurden 12 mit der Wiederholungsreaktion von Mantoux und 13 nach Pirquet untersucht (Tab. 2, 4 und 17). Von den erstgenannten 12 Fällen wies kein einziger Abschwächung auf, zweimal wurde Gleichbleiben und 10mal Steigerung festgestellt. Bei der Pirquet-Prüfung ergab sich bei Verwendung der 24-Std.-Werte einmal Abschwächung, 4mal Gleichbleiben und 8mal Steigerung. Die Anzahl der untersuchten Fälle war freilich gering, doch weist das Resultat namentlich in der wiederholten Mantoux-Reaktion darauf hin, dass inaktive Tuberkulose im Gegensatz zur aktiven Lungentuberkulose im allgemeinen eine Sensibilitätssteigerung aufweist.

Pleuritis exsudativa. An exsudativen Pleuritiden wurden 63 untersucht, davon 48 nach Mantoux und 15 nach Pirquet (Tab. 2, 4 und 17). Es waren nur zu einem geringen Teil frische Fälle, denn nur selten kamen sie unmittelbar nach der Erkrankung in das Kriegslazarett, wo die Tuberkulinwiederholungsprüfungen ausgeführt wurden. Die meisten waren vorher in einem Feldlazarett oder in anderen Militärlazaretten behandelt worden. Von den mit erneuter Mantoux-Reaktion untersuchten 48 Pleuritisfällen

zeigten 2, d.h. 4 %, Abschwächung, 12, d.h. 25 %, Gleichbleiben und 34, d.h. 71 %, Steigerung. 6 der Fälle waren innerhalb eines Monats vor Beginn der Mantoux-Versuche erkrankt; in einem derselben fand man als Resultat Gleichbleiben, in den übrigen 5 Steigerung. Die restierenden 42 Pleuritisfälle waren älteren Datums; die betreffenden Symptome hatten sich innerhalb eines Zeitraums von 6 Monaten vor dem Beginn der Mantoux-Untersuchungen gezeigt. Im erneuten Mantoux ist also das Verhalten bei Pleuritis exsudativa ein ähnliches wie bei inaktiver Tuberkulose oder bei Gesunden.

Mit erneuter Pirquet-Reaktion wurden 15 Fälle von Pleuritis exsudativa untersucht; 5 derselben zeigten bei Verwendung der 24-Std.-Werte Abschwächung, 4 Gleichbleiben und 6, d.h. 40 %, Steigerung.

Auch bei der wiederholten Pirquet-Reaktion kommt also Steigerung häufiger vor als bei aktiver Lungentuberkulose, obwohl der Unterschied kleiner ist als der bei entsprechender Mantoux-Prüfung gefundene.

Man kommt also zu dem Schluss, dass die Fälle von Pleuritis exsudativa sich bei der Tuberkulinwiederholungsreaktion ähnlich verhalten, wie die Fälle von inaktiver Tuberkulose.

Sonstige Tuberkulose. Diese Fälle sind nur 8 an der Zahl, nämlich 7 mit Tuberculosis lymphoglandularum und einer mit Peritonitis tuberculosa. Nach Mantoux wurden 2 von ihnen untersucht, und zwar der eine mit Abschwächung, der andere mit gleichem Resultat. Nach Pirquet untersuchte ich 6 Fälle und fand dabei dreimal Abschwächung, zweimal Gleichbleiben und nur einmal Steigerung.

Wie die Tuberkulinwiederholungsreaktion darlegt, scheinen die Resultate bei sonstiger Tuberkulose von ähnlicher Art zu sein, wie bei Lungentuberkulose.

Suspekte Tuberkulose. Es gab im ganzen 14 Fälle, wo in Ermangelung genügender Beobachtungsdauer oder aus irgendeinem anderen Grunde die Diagnose nicht gesichert werden konnte. 9 von diesen Fällen wurden nach Mantoux geprüft, wobei zweimal Abschwächung, dreimal Gleichbleiben und viermal Steigen der Sensibilität auftrat. Die Zahl der nach Pirquet Untersuchten war 5; einer derselben zeigte Abschwächung, die übrigen 4 Steigerung.

Andere Krankheiten. Die Diagnosen der zu dieser Gruppe gehö-

renden Krankheitsfälle lauteten: Bronchitis, Laryngitis acuta, Residua post pneumoniam, Infectio acuta, Myocarditis, Angina tonsillaris, Pro observationem, Anaemia simplex, Bronchiectasiae. Von diesen Fällen wurden 9 mit Mantoux, 13 mit Pirquet erneut untersucht.

Die Mantoux-Wiederholungsprüfung ergab einmal Abschwächung, einmal Gleichbleiben und in den übrigen 7 Fällen Steigerung.

Die mit erneutem Pirquet untersuchten, an nicht tuberkulösen Krankheiten Leidenden waren 13 an der Zahl, davon 1 Fall mit Abschwächung, 2 Fälle mit gleichem Resultat und 10 mit Steigerung.

Die Anzahl der untersuchten Fälle war allerdings sehr gering, doch deutet das Resultat deutlich an, dass die mit nicht tuberkulösen Krankheiten behafteten Fälle meistens bei der Tuberkulinwiederholungsreaktion ein Steigen der Sensibilität erkennen lassen.

Gesunde. 278 Gesunde wurden erneut geprüft, davon 83 mit Mantoux und 195 mit Pirquet (Tabelle 2, 4, 17).

Von den 83 mit Mantoux Wiederholungsreaktion untersuchten Gesunden zeigten 6, d.h. 7.2 %, Abschwächung, 8, d.h. 9.6 %, Gleichbleiben und 69, d.h. 83.2 %, Steigerung.

Alle Gesunde, welche entweder die Mantoux- oder die Pirquet-Wiederholungsprüfung durchmachten, wurden im Verlauf der Tuberkulinuntersuchungen mit der in unserem Heer gebräuchlichen Röntgen-Schirmbildphotographie untersucht, oder, falls es sich um Krankenhauspersonal handelte, fand Röntgendurchleuchtung statt; also haben diese Untersuchungen die Möglichkeiten vermindert, dass es unter den Versuchspersonen mehrere vielleicht unerkannt gebliebene Lungentuberkulöse gegeben haben könnte. Ausserdem bestand ja der grösste Teil des Materials aus Wehrpflichtigen, die schon bei der Einberufungs- und Rekrutenuntersuchung kurz vorher ärztlich besichtigt worden waren, bei welcher Gelegenheit gerade dem Vorkommen einer eventuellen Tuberkulose grosse Aufmerksamkeit geschenkt wird.

Die mit Pirquet erneut geprüften 195 Gesunden zeigten 26mal, d.h. in 13.3 %, Abschwächung, 63mal, d.h. in 32.3 %, gleiches Resultat und 106mal, d.h. in 54.4 %, Steigerung. Die Pirquet-Wiederholungsreaktion ergibt für die Gesunden prozentuell so wenig Steigerungsfälle, dass der Unterschied im Vergleich zur

aktiven Lungentuberkulose, die 25 % Steigerungen aufwies, bei weitem nicht so gross ist, wie beim entsprechenden Mantoux.

Die Tuberkulinwiederholungsprüfungen an Gesunden führen zu folgenden Schlüssen: Die Wiederholungsprüfung nach Mantoux ergibt Steigerung in 83.2 % der Fälle, nach Pirquet nur in 54.4 %. Der Unterschied zwischen den Fällen von aktiver Lungentuberkulose ist in den Pirquet-Resultaten so gering, dass diese Wiederholungsreaktion bei Diagnostizierung der aktiven Tuberkulose eine recht kleine Rolle spielt.

BCG-Impfung (Tabelle 2, 4 u. 17). Gegen Tuberkulose geimpft waren 116 Wehrpflichtige, die zwei Monate nach der Schutzimpfung der Tuberkulinwiederholungsprüfung unterzogen wurden. Die Prüfung erfolgte in 67 Fällen nach Mantoux, in 49 nach Pirquet.

Von den 67 erneut nach Mantoux untersuchten BCG-Geimpften zeigten 33, d.h. 49.3 %, Abschwächung, 11, d.h. 16.4 %, Gleichbleiben und 23, d.h. 34.3 %, Steigerung. Die Prozentzahl der Steigerungen war also bei der erneuten Mantoux-Prüfung ungefähr ebenso hoch wie in den Fällen von aktiver Lungentuberkulose, und Abschwächung kam so häufig vor, dass man in keinem der drei Lungentuberkulosestadien eine so hohe Prozentziffer findet.

49 gleichzeitig BCG-Geimpfte, die 2 Monate später mit Pirquet erneut geprüft wurden, hatten 13mal, d.h. in 26.5 %, Abschwächung, 23mal, d.h. in 47.0 %, Gleichbleiben und 13mal d.h. in 26.5 %, Steigerung aufzuweisen. Nach Pirquet tritt somit Abschwächung der Sensibilität prozentuell etwas seltener auf, als bei aktiver Lungentuberkulose. Bei den BCG-Geimpften war also das Steigerungsprozent ungefähr dasselbe, wie bei aktiver Lungentuberkulose.

Zusammenfassend kann von den Resultaten der Tuberkulinwiederholungsreaktion an den 2 Monate vorher BCG-Geimpften erwähnt werden, dass man ungefähr zu demselben Ergebnis kam, wie in den Fällen von aktiver Lungentuberkulose, nur war nach Mantoux die Abschwächung noch häufiger, als bei Lungentuberkulose.

Besprechung und Schlussbetrachtung.

Ich habe in betreff der benutzten Technik sowohl in der Ausführungsweise als auch bei Ablesung der Resultate nach möglichster Einfachheit gestrebt, damit das Verfahren bessere Voraus-

setzungen hätte, auch allgemeinere Verwendung zu finden. Teils unter dem Zwang der Verhältnisse, teils um möglichst bald eine Wahrscheinlichkeitsantwort auf die Frage, ob aktiv oder inaktiv, zu erhalten, wählte ich meistens für die Tuberkulinwiederholungsprüfung das Intervall 5 Tage. Etwas besser wären auch nach meiner Erfahrung die Resultate bei Verwendung eines längeren Intervalls geworden.

Betrachtet man die Ergebnisse der Mantoux-Wiederholungsprüfung in den einzelnen Krankheitsgruppen (Tabelle 4), so scheint das Resultat schlecht zu sein, wenigstens wenn man ebenso wertvolle Befunde erwartet, wie die Wassermann-Reaktion sie bei der Luesdiagnostik liefert. Aber diese Erwartung und eine solche Fragestellung sind falsch infolge des Charakters der Tuberkulosekrankheit. Bei einem Tuberkulinpositiven kann beispielsweise ein durch exo- oder endogene Superinfektion hervorgerufener Herd, der die Krankheit in Bewegung setzt, winzig klein sein und der Organismus sich eine Zeitlang gleichsam in einem labilen Gleichgewichtszustand befinden. Es ist möglich, dass der Herd ausheilt, ohne irgendwelche klinische Zeichen der Aktivität zu zeigen. Eine andere Möglichkeit ist die, dass das Gleichgewicht nach der anderen Seite überschlägt, der Prozess um sich greift und eine auch klinisch aktive Lungentuberkulose entsteht. Eine derartige Superinfektion, die ganz ohne klinische Symptome in den Lungen ausheilt, kann bei vielen tuberkulinpositiven Gesunden und gesund Verbleibenden auftreten; aber wenn ein solcher Prozess mitten in seiner Ausheilung durch eine Tuberkulinwiederholungsreaktion aufgedeckt würde, so würde man natürlich den Befund als ein von dieser Reaktion geliefertes »unspezifisches« Resultat ansprechen.

Betrachtet man andererseits bei aktiver Lungentuberkulose die hohe Prozentziffer der im erneuten Mantoux Steigerung darlegenden Fälle, so ist es möglich, dass auch sie nicht auf Ungenauigkeit der Methode allein zurückgeführt zu werden braucht. Stellen wir uns eine weiter fortgeschrittene Lungentuberkulose als die im vorigen Beispiel erwähnte vor, so kann man annehmen, dass die Tuberkulinempfindlichkeit zum Gleichgewicht der Antigen-Antikörper des Organismus in Beziehung steht. Wird bei aktiver Lungentuberkulose eine Tuberkulineinspritzung verabfolgt, so kann dies eine schwache oder starke Reaktion zur Folge haben. Befindet sich die Lungentuberkulose in einem ernsten Stadium und enthält

das Blut einen Antigenüberschuss im Vergleich zu den Antikörpern, so besitzt auch der Organismus nicht genügend Möglichkeiten eine starke Reaktion hervorzurufen, und im Endstadium der Krankheit kann sogar die Tuberkulinprobe negativ ausfallen. Die Antigenmenge im Blut braucht nicht nur im Endstadium der Krankheit, sondern kann auch sonst stark erhöht sein, z. B. während eines hämatogenen Schubs. Wenn es sich um die Tuberkulinwiederholungsprüfung handelt, hat man Abschwächung oder ein gleiches Resultat zu erwarten, wenn ein Antigenüberschuss im Organismus existiert. Dann ist das verabfolgte Tuberkulin nicht imstande, den Organismus so zu sensibilisieren, dass er auf eine nach gewisser Zwischenzeit verabreichte neue, ebenso grosse Tuberkulinmenge stärker reagieren würde. Der Organismus besitzt sozusagen nicht genug Kraft dazu. Aber sogar bei weit ausgehnter Lungentuberkulose kann der Antikörperüberschuss zeitweilig gross sein oder ist der Organismus auf die Fähigkeit eingestellt, rasch eine grosse Antikörpermenge zu produzieren; dann reagiert der Organismus im Tuberkulinwiederholungsversuch in derselben Weise wie bei inaktiver Tuberkulose, und dann hat die vorige Tuberkulinimpfung den Organismus dermassen sensibilisiert, dass die folgende Tuberkulinimpfung eine stärkere Reaktion als die erste liefert.

Von den vorerwähnten Annahmen ausgehend, lässt es sich auch leicht erklären, warum man bei Pleuritis im wiederholten Tuberkulinversuch so oft Steigerung findet (Mantoux 71 % und Pirquet 40 %). Auch die erneute Tuberkulinprüfung zeugt somit vielleicht ihrerseits von der Gutartigkeit der Pleuritiskrankheit, die auch ich bei meiner klinischen Arbeit während des Krieges vielfach kennen gelernt habe.

Wie ist nun der Umstand zu erklären, dass die Tuberkulinwiederholungsreaktion bei BCG-Geimpften ungefähr das gleiche Verhalten aufweist, wie bei Kranken mit aktiver Lungentuberkulose? Wahrscheinlich hängt es davon ab, dass der Reiz des Impfstoffs 2 Monate nach der Impfung hinreichend kräftig gewesen ist, um den Tuberkulinversuch positiv zu machen, aber eine zu kurze Zeitdauer oder ein ungenügender Impfreiz oder aber beide Ursachen zusammen haben bewirkt, dass der Organismus nicht dasselbe Stadium erreicht hat wie ein inaktiver Tuberkulosefall.

Der Umstand, dass der erneute Pirquet schlechtere Resultate geliefert hat, als der erneute Mantoux, beruht wahrscheinlich darauf, dass die Pirquetsche Reaktion gröber und ungenauer ist als die Mantoux'sche. Bei Pirquet schwankt ganz unwillkürlich in den einzelnen Fällen und bei verschiedenen Gelegenheiten die Stärke der Bohrung, und ausserdem kann die Menge des wirksamen Tuberkulins das eine Mal grösser sein als das andere, was sich absolut nicht vermeiden lässt. Infolgedessen kann vermutlich die Pirquetsche Wiederholungsreaktion niemals ebenso grossen Wert besitzen, wie die Mantoux'sche.

Schlussfolgerungen.

Es wurden mit der Tuberkulinwiederholungsreaktion insgesamt 781 tuberkulinpositive Personen untersucht, und zwar 385 nach Mantoux und 396 nach Pirquet. Die Resultate lassen sich folgendermassen zusammenfassen.

Bei Versuchen, zu erforschen, welches Intervall in der Tuberkulinwiederholungsprobe das brauchbarste wäre, ergab es sich, dass 14 Tage im erneuten Mantoux vielleicht etwas bessere Resultate liefern als 5 Tage, aber dass im erneuten Pirquet 14 Tage ganz deutlich eine günstigere Zwischenzeit bilden, als 5 Tage.

Im erneuten Mantoux ist es vorteilhafter, den von den 48-stündigen Werten dargelegten Unterschied als Beurteilungsgrundlage zu benutzen, während im erneuten Pirquet die Unterschiede der 24-stündigen Werte entschieden bessere Resultate geben.

Ein Gleichbleiben und sogar schon eine leichte Abschwächung der Sensibilität sind bei der Tuberkulinwiederholungsreaktion charakteristisch für die aktive Lungentuberkulose. Eine kräftigere Steigerung spricht gegen Aktivität.

Gleiches Resultat und Abschwächung kommen bei fieberhafter Lungentuberkulose häufiger vor als bei fieberfreier.

Desgleichen sind bei erneuter Tuberkulinprüfung Gleichbleiben und Abschwächung gewöhnlichere Erscheinungen in solchen Lungentuberkulosefällen, wo die Senkungsgeschwindigkeit der roten Blutkörperchen (nach Westergren) hoch ist, als in Fällen mit niedriger Senkungsgeschwindigkeit.

Die Fälle von Pleuritis exsudativa ergeben bei der Tuberkulinwiederholungsprüfung zum grössten Teil Sensibilitätssteigerung.

Das Ergebnis der erneuten Tuberkulinreaktion bei Gesunden ist in der Regel Steigerung.

Zwei Monate vor der Tuberkulinwiederholungsprüfung BCG-Geimpfte verhalten sich bei dieser Prüfung ungefähr ebenso wie Personen mit aktiver Lungentuberkulose; diese Feststellung trägt ihrerseits dazu bei, die Ansicht von der Vorteilhaftigkeit der BCG-Impfung immunobiologisch zu stützen.

Die Tuberkulinwiederholungsreaktion bringt bei der klinischen Untersuchung von Lungentuberkulosefällen ein neues immunobiologisches Symptom zum Vorschein, welches durch seinen, allerdings relativen Wert einen weiteren Beitrag zur Aktivitätsdiagnostik der Lungentuberkulose liefert.

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Recherches sur la physiopathologie des hématies.

I. Nouvelles techniques pour l'étude et la distinction des hématies granuleuses.

Par

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(Ce travail est parvenu à la rédaction le 6 Septembre 1943).

Malgré la littérature importante consacrée jusqu'à présent à l'étude des hématies granuleuses, nous ne possédons à leur sujet que des notions d'une précision et d'une exactitude insuffisantes. Les structures granuleuses des hématies sont souvent à la limite de la vision microscopique. Leur visibilité dépend étroitement de contingences d'ordre technique. Les caractères morphologiques de ces structures ne permettent de les distinguer que dans des cas limites. De plus, les procédés classiques de coloration ne sont pas rigoureusement spécifiques.

L'observation microscopique sur fond noir n'a guère été utilisée jusqu'ici en biologie que pour l'étude d'organismes vivants observés à l'état frais. Les résultats obtenus ont été souvent médiocres et difficiles à interpréter. Au contraire, l'examen sur fond noir de préparations fixées et colorées constitue un procédé très sûr, applicable dans des conditions excellentes à l'étude des hématies granuleuses. Il nous a permis d'en assurer une identification précise. Sur cette base solide, il devient possible d'aborder une étude méthodique de la physiopathologie des hématies, considérée notamment du point de vue du métabolisme de l'hémoglobine.

1. Observation des réticulocytes.

A. — *Nécessité d'une technique précise.* — De nombreuses méthodes de coloration des réticulocytes par des substances basiques ont été préconisées. Citons notamment les techniques de Rosin et Bibergeil (bleu de crésyl brillant et bleu de toluidine) (49), Chauffard et Fiessinger (pyronine-vert de méthyle) (2), Hoffmann (bleu de crésyl et rouge neutre) (28) Widal, Abrami et Brule (bleu d'Unna, bleu d'Azur) (65) Cesaris-Demel (1), Cunningham (4), Friedlander et Wiedemer (17), Holboll (29), Seyfarth (58), Krumbhaar (38) Heilmeyer et Trachtenberg (23), Cook (3), Osgood et Wilhelm (46), Hirschfeld et Moldawsky (27), Ederle (11), Schleicher (52), Heilmeyer et Oortgiese (25), Leitner (41) Larsen et Skadhauge (39) (bleu de crésyl brillant avec éventuelle coloration complémentaire; observation sur le frais en chambre humide ou sur frottis desséché).¹

Ces techniques ne diffèrent en général que par des détails de peu d'importance. Les résultats obtenus par ces auteurs dans des conditions analogues sont néanmoins fort divergentes: les valeurs trouvées pour le taux moyen de réticulocytes chez le sujet humain normal, d'âge adulte, en sont une preuve: elles vont de 0.3 % à 30 %. Les résultats dépendent trop étroitement de conditions accessoires (qualité du frottis, durée de la coloration, concentration des solutions colorantes, etc.) Widal, Abrami et Brule, Moldawsky Heilmeyer et Oortgiese, Schleicher, Osgood et Wilhem, Cook, Krumbhaar, Friedlander et Wiedemer colorent les hématies en présence de citrate ou d'oxalate alcalins. Nous verrons que ces sels altèrent gravement la coloration des réticulocytes. Des résultats aussi variables et inconstants ne se prêtent évidemment pas à des études précises.

B. — *Description de la technique.* — Notre méthode permet de combler cette lacune. Elle donne des résultats précis et réguliers; grâce à l'élimination de la plupart des causes d'erreurs signalées précédemment. Notre méthode de coloration est celle de Heilmeyer et Trachtenberg (23) légèrement modifiée.

¹ Voir également Dameshek (5), Fiessinger et Laur (13), Franke (15), Franke (16), Kämmerer (34), Johns (31).

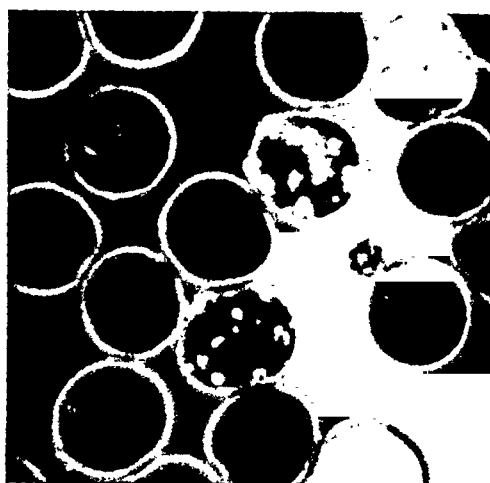
Dans un godet en paraffine, on recueille directement quatre gouttes de sang prélevées au lobe de l'oreille ou à l'extrémité du doigt. On y ajoute quatre gouttes de solution à 1 % de bleu de crésyl brillant dans du liquide physiologique. Si le sang est pauvre en éléments figurés, il faut réduire la quantité de colorant à une ou deux gouttes. On mélange ensuite avec un agitateur paraffiné et on laisse colorer en chambre humide pendant vingt minutes. On remet très soigneusement les hématies en suspension dans le liquide; on fait des frottis très réguliers et minces à partir du mélange et on laisse sécher. Pas de fixation. Cette technique permet d'obtenir sans peine de très beaux étalements. On peut sans inconvénient surcolorer fortement. Les étalements peuvent être conservés pendant quelque temps à sec ou montés dans un milieu non réducteur.

L'observation sur fond noir se fait en général avec l'objectif à immersion à huile de cèdre. Si l'on utilise un objectif à immersion à l'eau ou un objectif à sec, il est indispensable de monter la préparation sous lamelle dans l'huile de cèdre.

Un condensateur à fond noir moderne d'ouverture numérique élevée est indispensable. Les instruments plus anciens (condensateur parabolique ou simple diaphragme central) ne peuvent suffire. La source lumineuse doit être d'une intensité spécifique élevée. Les petites lampes à incandescence à bas voltage sont les plus commodes. Tout objectif à immersion d'ouverture numérique élevée doit être diaphragmé.

Les photographies ci-jointes (fig. 1) ont été obtenues avec le condensateur cardioïde de Zeiss et avec l'objectif achromatique à immersion, 90 \times , de même marque, diaphragmé jusqu'à l'ouverture numérique de 0.95.

Il est nécessaire de travailler avec une grande propreté. Si l'on observe sur fond noir un frottis de sang coloré comme nous venons de l'indiquer, les hématies mûres apparaissent colorées en rouge vineux; les leucocytes en jaune orangé; les plaquettes en bleu-vert; la substance réticulo-filamenteuse des réticulocytes est colorée en jaune-vert très brillant. Les poussières apparaissent colorées en blanc; les hémocoques en blanc jaunâtre; les précipités de colorant en rouge rubis.



a

Réticulocytes, hématies, plaquette.



b

Leucocyte.

Fig. 1.

Avantages de cette technique.

a) Identification certaine des réticulocytes sans confusion ou omission possibles. Nous verrons plus loin que la coloration de la S. R. F. est rigoureusement spécifique et qu'elle n'est donnée par aucune autre espèce de granulation intraglobulaire.

Nous constatons ici un phénomène remarquable: un colorant peu ou pas métachromatique sur fond clair donne sur fond noir une série de superbes teintes métachromatiques.

b) Grande commodité d'observation; technique beaucoup moins fatigante que les méthodes courantes;

c) grande fidélité: résultats indépendants de la qualité du frottis et de l'intensité de la coloration.

d) Mise en évidence de formes réticulocytaires très évoluées, où la S. R. F. très ténue est complètement invisible sur fond clair.

Les taux de réticulocytes obtenus sont donc plus élevés que ceux trouvés par l'ensemble des méthodes classiques. Ils sont, chez l'homme normal des deux sexes, compris dans la grande majorité des cas entre 15 ‰ et 25 ‰.

Les structures observées sur fond noir correspondent bien aux réticulocytes. En effet:

a) on peut aisément vérifier cette identité en observant un même élément successivement sur fond clair, sur fond noir, au moyen d'un condensateur alternatif;

b) dans les réticulocytes riches en S. R. F., la disposition morphologique de celle-ci est la même sur fond noir et sur fond clair;

c) la coloration métachromatique jaune-vert de la S. R. F. est, nous le verrons, rigoureusement spécifique.

Les formes invisibles sur fond clair sont bien des réticulocytes; en effet:

a) les granulations de ces éléments présentent les caractères spécifiques d'évolution et de coloration de la S. R. F.

b) Nous verrons dans un prochain article relatif à l'évolution des réticulocytes, que les formes riches en S. R. F., visibles sur fond clair, évoluent vers des formes invisibles sur fond clair, mais visibles seulement sur fond noir (Effet ultra-microscopique).

C. — Influence des anticoagulants sur la coloration des réticulocytes.

Les expériences in vitro exigent l'emploi de sang rendu incoagulable. Klima et Rosegger (37) signalent que l'addition de citrate sodique au sang étudié entraîne une altération de la coloration de la substance réticulo-filamenteuse.

Nous avons étudié l'influence sur la coloration des réticulocytes des solutions anti-coagulantes suivantes:

solution aqueuse de citrate sodique à 10 %
» » d'oxalate » » 10 %
» » de fluorure » » 5 %
» de Liquoïde de Roche à 800 mg $\frac{0}{100}$ dans le liquide physiologique.

Un même sang hépariné, réparti dans plusieurs godets, est respectivement additionné de 20 % de chacune de ces solutions. Un témoin est représenté par du sang hépariné tel quel. Après coloration et étalement, nous procédons à une numération comparative des réticulocytes. Le tableau ci-dessous donne les résultats de 10 expériences semblables.

Tableau n° 1.

	Héparine	Citrate Na	Fluorure Na	Oxalate Na	Liquoïde
I	20	11	impossibles à compter	19	17
II	32	15	13	20	28
III	36	19	10	12	9
IV	92	33	22	14	50
V	22	24	impossibles à compter	8	18
VI	40	38	24	8	32
VII	33	20	18	12	16
VIII	28	26	18	12	10
IX	28	10	10	8	22
X	14	14	7	4	12

L'oxalate, le citrate et le fluorure sodique, ainsi que le liquoïde Roche, altèrent donc considérablement la coloration des réticulocytes. La structure de ces éléments est également modifiée. L'héparine seule n'altère pas cette coloration; elle donne les mêmes résultats que ceux que l'on obtient avec du sang prélevé directement sur paraffine. La structure de la substance réticulofilamenteuse n'est pas modifiée. Les autres anti-coagulants donnent des résultats inférieurs.

D'après Heath et Daland (22) le glucose en solution à 8 % gêne considérablement la coloration des réticulocytes. Nous n'avons rien observé de semblable dans de nombreuses expériences sur du sang glucosé.

*D. — Divers colorants de la substance réticulo-filamenteuse.
Leur comparaison.*

Unger (61) préfère au bleu de crésyl brillant le violet cristal; il emploie aussi le sulfate de bleu de Nil, l'Azur et le bleu de méthylène. Klima et Rosegger (37) préfèrent le sulfate de bleu de Nil.

Sur fond noir, le bleu de crésyl brillant se montre de beaucoup le meilleur colorant des réticulocytes: ceux-ci sont mis en évidence en beaucoup plus grand nombre et la S. R. F. apparaît avec une netteté parfaite. D'accord avec Unger (61), nous avons vérifié que

sa répartition varie avec le colorant utilisé. Seul, le bleu de crésyl brillant donne de la métachromasie. Dans ce tableau comme dans la suite, les taux sont exprimés en éléments sur 1000 hématies.

Nous avons obtenu sur fond noir les colorations suivantes: rouge neutre: blanc verdâtre clair — sulfate de bleu de Nil: jaune brun clair — violet cristal: orangé pâle, réticulocytes peu visibles — Bleu Victoria: blanchâtre — Violet de Méthyle; jaunebrun Bismarck: blanchâtre; éléments peu visibles — Bleu de toluidine: rouge pâle, réticulocytes très peu visibles.

Nous considérons donc que la technique la plus sûre et la plus sensible pour l'étude des réticulocytes est l'examen sur fond noir de préparations de sang pur ou hépariné, coloré au bleu de crésyl brillant, étalé et séché, sans fixation.

Dans la marge des variations rencontrées dans nos expériences, le pH et la pression osmotique n'influencent pas la mise en évidence des réticulocytes.

E. — *Formule réticulocytaire.*

Plusieurs auteurs ont été établis des classifications des réticulocytes basées sur les caractères morphologiques et l'abondance de la substance réticulo-filamenteuse: Engel (12), Gawrilow (20), Heilmeyer et Trachtenberg (23), Heilmeyer et Westhäuser (24), Mesirca (43). On sait que la S. R. F. est d'autant plus ténue que les réticulocytes sont plus âgés, moins éloignés de l'état d'hématie mûre. Naegeli (45) Cesaris-Demel (1), Lee, Minot et Vincent (40), Denecke (6), Gawrilow (19), Engel (12), Moldawsky (44), Sevfarth et Jürgens (57).

Nous classons les réticulocytes en 5 groupes en nous basant uniquement sur la quantité progressivement décroissante de S. R. F. qu'ils contiennent et non sur des caractères morphologiques peu précis et sans grande signification. L'observation sur fond noir avec un grossissement élevé (2000 \times) permet un véritable dosage semi-quantitatif de cette substance.

Cette technique présente divers avantages nécessaires à une étude précise:

a) grande indépendance du résultat vis-à-vis des variations de la coloration, de la qualité des préparations et de la précision de l'observation,

b) réduction, dans une mesure suffisante des facteurs subjectifs d'appréciation.

c) possibilité de diviser l'ensemble du processus évolutif des réticulocytes en stades bien définis d'importance à peu près égale.

Le groupe I correspond aux normoblastes, le gr. V aux hématies ne contenant plus que quelques traces de substance réticulo-filamenteuse (fig. 2). Les cinq groupes que nous considérons ne sont naturellement pas superposables aux groupes décrits par d'autres auteurs (voir ci-dessus).

Il est évident que cette division en groupes est arbitraire. Il suffit à la validité des résultats obtenus à partir de ces formules, que toutes les évaluations expérimentales se fassent sur la même base de répartition. De la sorte, d'éventuelles variations individuelles, d'un observateur à l'autre, n'auront par elles-mêmes aucune conséquence quant à la validité des raisonnements expérimentaux (voir articles ultérieurs).

2. Observations des ponctuations basophiles des hématies.

La technique d'examen sur fond noir convient parfaitement à l'observation des ponctuations basophiles des hématies, surtout fréquentes dans l'intoxication par le plomb. La coloration est celle de Manson-Schwartz (53 à 56) au bleu de méthylène. Pour le reste, les détails techniques d'observation sur fond noir ont été suffisamment exposés plus haut. Dans ces conditions, nous trouvons chez l'individu normal un taux d'hématies à ponctuations basophiles élevé, atteignant 8 et 14 $\frac{0}{100}$.

Ces éléments sont normalement présents dans la majorité des cas. Sur fond noir, les hématies et les granulations apparaissent colorées en brun. Les ponctuations sont réparties très régulièrement dans les stromas (fig. 3); toutes les ponctuations d'une hématie déterminée sont, en général, de grosseur analogue. Elles sont d'autant plus nombreuses qu'elles sont plus fines. Elles sont le plus souvent trop petites pour être visibles sur fond clair. Sur fond noir, elles ne peuvent échapper et leur recherche est beaucoup moins fatigante que par les méthodes d'examen sur fond clair. Nous avons contrôlé l'identité des éléments visibles sur fond noir comme nous l'avons fait pour les réticulocytes.

Dans certains états pathologiques ne comportant pas d'intoxication par le plomb, nous avons rencontré des taux très élevés d'hématies à ponctuations basophiles. (Exemples: 350 ‰ dans un cas d'ictère hémolytique acquis; 51 ‰ dans un cas de nephrite azotémique; 35 ‰ dans une hémorragie). Mais, dans tous ces cas, les granulations étaient très fines et invisibles sur fond clair.¹

La question des rapports existant entre réticulocytes et hématies à ponctuations basophiles a fait l'objet de travaux assez nombreux. Les conclusions sont opposées.

Widal, Abrami et Brule (65) concluent à la non identité de ces éléments. D'après ces auteurs on trouve toujours des réticulocytes chez l'homme normal, mais pas d'hématies à granulations basophiles. Ils observent dans un cas d'ictère hémolytique (63, 64) 65 % d'hématies granuleuses; colorant après fixation, ils n'observent plus aucun élément granuleux. Pour Schilling, (50) les hématies à ponctuations basophiles sont des formes pathologiques d'hématies jeunes; elles représentent des réticulocytes modifiés, altérés. D'après cet auteur, le bleu de crésyl brillant colore indifféremment les deux espèces d'éléments. Schilling prétend observer tous les intermédiaires entre les deux structures. P. Dustin Jr. (10), suivant dans des conditions déterminées les modifications de ces structures, les voit se transformer l'une dans l'autre.²

Une série d'examen de sang pratiqués d'après nos techniques dans des circonstances diverses nous conduisent à admettre la non identité des réticulocytes et des hématies à ponctuations basophiles.

Les caractères de coloration différents ne suffisent pas à prouver cette non identité. Il se pourrait que substance réticulo-filamenteuse et ponctuations basophiles correspondent à une même substance révélée sous des aspects dissemblables après action de réactifs différents.

¹ Haack (Diss. Rostock 1929) a appliqué l'observation sur fond noir à l'étude des hématies à ponctuations basophiles. Il emploie une coloration au bleu de toluidine d'après Litten-Süssmann. Par suite des circonstances actuelles, nous n'avons pu obtenir aucune indication sur ces techniques. Nous savons seulement que les taux d'hématies à ponctuations basophiles trouvées par ces auteurs dans l'intoxication par le plomb sont analogues à ceux trouvés sur fond clair.

² Consulter dans un article de H. Günther (21) le résumé l'autres travaux relatifs à cette question, ainsi que des indications bibliographiques complémentaires.

a) le tableau ci-dessous contient les résultats de numérations comparatives de ces éléments pratiquées dans 52 cas normaux ou pathologiques à l'exclusion de l'intoxication saturnine. (Taux exprimés en éléments sur mille).

Tableau n° 2.

No	Réticul.	Ponct. bas.	No	Réticul.	Ponct. bas.	No	Réticul.	Ponct. bas.
1	35.7	16	18	18	6	35	36	4
2	6	5	19	53	40	36	92	48
3	508	350	20	19	16	37	22	0.5
4	31.8	6	21	18	11	38	28	5
5	0	7	22	10	5	39	8	5
6	14.4	0.5	23	14	6	40	14	4
7	17	0	24	33.1	7	41	40	1
8	19	8	25	13	6	42	45	20
9	29	51	26	0.5	0	43	9	0
10	12	2	27	14	0	44	26	6
11	344	64	28	69	2	45	14	0
12	20	11	29	16.5	0	46	24	0
13	29	6	30	60	8	47	35	0
14	15.4	14	31	195	35	48	42	1
15	22.4	3	32	88	18	49	37	1
16	19.6	2	33	28	0	50	19	0
17	25	8	34	28	1	51	36	0
						52	15	0

Contrairement aux conclusions de Widal, Abrami et Brule, les hématies à ponctuations basophiles sont présentes dans la plupart des cas. Certaines réticulocytoses élevées coïncident avec un taux élevé d'hématies, à ponctuations basophiles; (n:o 1, 3, 11, 19, 31, 32, 36) cette relation n'est pas constante. Nous avons d'autre part trouvé dans les deux cas d'ictère hémolytique un taux exceptionnellement élevé de ces éléments (n:o 3 et 32), contrairement à Widal, Abrami et Brule. L'absence de ponctuations basophiles peut coïncider avec une réticulocytose élevée; par contre, on peut rencontrer des ponctuations basophiles dans un sang totalement privé de réticulocytes (n:o 5 anémie aplastique). En dépit des rectifications que l'amélioration de technique nous conduit à lui apporter, l'argument de Widal, Abrami et Brule conserve toute sa valeur.

b) Klima et Seyfried (36) ont signalé l'existence de granulations basophiles dans les éléments rouges de la moëlle. Nous avons examiné sur fond noir des frottis de moëlle sternale colorée par la méthode de Manson-Schwartz. L'examen sur fond noir permet seul la mise en évidence des fines granulations basophiles dans les érythrocytes nucléés. Sur fond clair, beaucoup de ponctuations basophiles sont dissimulées par l'intense coloration de fond du protoplasme. Il n'est dès lors pas possible de conclure avec certitude à la présence ou à l'absence de fines ponctuations dans un normoblaste déterminé. Sur fond noir, le protoplasme des normoblastes apparaît optiquement vide (éléments à noyau pycnotique) et les ponctuations basophiles brillantes se détachent sur le fond sombre du protoplasme avec une grande netteté (fig. 3). Dans ces conditions, une faible fraction des normoblastes contiennent des ponctuations basophiles. On sait que la substance réticulo-filamenteuse est présente dans tous les normoblastes.

c) au cours de la conservation du sang à l'étuve à 37° C. nous avons observé (voir communication ultérieure) une augmentation nette du nombre des hématies à ponctuations basophiles ainsi que de la grosseur de cette dernière. Au contraire, les réticulocytes vont en diminuant de nombre en même temps que la substance réticulo-filamenteuse décroît. Ce phénomène d'augmentation des ponctuations basophiles n'est pas constant.

Nous ne pouvons nous rallier à l'assertion de Schilling (50) d'après laquelle le bleu de crésyl brillant colore à la fois la substance réticulo-filamenteuse et les ponctuations basophiles; en effet:

a) une bonne coloration au bleu de crésyl brillant ne montre jamais la répartition parfaitement régulière si caractéristique des ponctuations basophiles.

b) dans un sang contenant 7⁰/₁₀₀ d'hématies à ponctuations basophiles colorées par la méthode de Manson-Schwartz et observées sur fond noir, la coloration au bleu de crésyl brillant combinée à l'examen sur fond noir a révélé l'absence complète de réticulocytes (tableau n° 2 — cas n° 5). Dans un autre cas (n° 9) (tableau n° 2) on trouve plus d'hématies à p.b. que de réticulocytes.

Par ailleurs, la présence en quantité plus ou moins grande, parfois notable, de ponctuations basophiles chez beaucoup d'individus paraissant normaux, ne permet pas de reconnaître à ces éléments un caractère fondamentalement et nettement pathologique.

Dans certains états s'accompagnant d'une stimulation de la moëlle, révélée par une crise réticulocytaire avec déplacement de la formule réticulocytaire vers la gauche (formes jeunes) on peut observer une augmentation du taux des hématies à ponctuations basophiles. Bien que ce dernier phénomène ne soit pas constant, on peut admettre qu'une relation existe, *dans une mesure limitée* entre les deux espèces d'éléments, en ce sens que, dans les états de grande stimulation de la moëlle, une proportion plus élevée d'hématies contiennent des ponctuations basophiles. Cette relation ne peut se comprendre dans le sens d'une simple identité de nature. Il s'agit de processus différents. Dans l'état actuel de nos connaissances, il n'est pas possible de donner des précisions sur les relations qui semblent exister entre eux.

3. Corps et granulations de Heinz.

En 1890, Heinz (26) observa dans le sang d'animaux intoxiqués par la phénylhydrazine une nouvelle espèce d'hématies granuleuses. Roehl, en 1890 (48) observa les mêmes structures basophile après intoxication par le dinitrophénol. On trouvera dans un article de P. Dustin Jr. (10) une liste des substances dont l'administration entraîne l'apparition de corps de Heinz.

Nous avons vu apparaître ces éléments dans le sang conservé à l'étuve pendant un temps suffisant. Il est possible que les granules observés par Huber (30) dans les sangs abandonnés pendant plusieurs jours, correspondent à des corps de Heinz. Cette fois encore, la technique d'observation sur fond noir, appliquée à des préparations colorées de ces éléments, présente de grands avantages. Les corps de Heinz colorés supravitalement par le bleu de crésyl brillant, le bleu de Nil ou le violet de méthyle présentent sur fond noir une très belle coloration brune, intense surtout avec le bleu de Nil. L'intensité de la coloration par le bleu de Nil a été signalée par Friedstein (18). Toutefois le bleu de crésyl brillant donne une belle coloration brun-rouge un peu moins intense mais plus fine. On considérerait jusqu'à présent que les corps de Heinz volumineux se rencontraient en petit nombre dans les hématies intéressées; ces caractéristiques morphologiques limitaient à des cas peu fré-

quents l'éventualité d'une confusion avec un réticulocyte. Les méthodes de coloration sont, remarquons-le, analogues, sinon identiques.

Nous avons constaté que la morphologie des corps de Heinz varie suivant le sujet humain ou l'animal en expérience et suivant la quantité de toxique. Les corps de Heinz obtenus par l'action de la phénylhydrazine *in vitro* sur le sang humain sont tantôt volumineux, d'une morphologie assez typique, tantôt plus petits et difficiles à distinguer des réticulocytes, quelle que soit la méthode sur fond clair utilisée. Chez l'animal (Chien) la disposition de cette structure basophile obtenue par l'action de la phénylhydrazine *in vitro* ou *in vivo*, ne permet absolument pas de la distinguer directement des réticulocytes, quelle que soit son abondance dans les hématies. Sur fond clair, les deux structures sont identiques, tant par leur morphologie que par leur coloration. L'examen sur fond noir permet une distinction directe, sûre et précise des deux éléments; une préparation colorée au bleu de crésyl brillant par la méthode décrite ci-dessus à propos des réticulocytes et observée sur fond noir montre les hématies colorées en rouge, la S. R. F. en vert-doré, les corps de Heinz en brun-rouge. Aucune différence de coloration n'est visible sur fond clair. Nous proposons d'appeler ces structures «granulations de Heinz». Un assez grand nombre de travaux ont pour but d'étudier les réticulocytes obtenus par l'administration de phénylhydrazine. Schilling (51) R. Duesberg (7, 8, 9) Tanzi (59, 60) Katchoura (35) Fiessinger et Laur (14), signalent dans ces conditions des réticulocytoses massives. Ces réticulocytoses ont été étudiées par Johnson et Berglund (32), Ritz (17) Gawrilow (20) Withby et Britton (62), Yang et Berglund (68) Massa (42), Wright et Payling (67) etc. Les phénomènes observés par ces auteurs doivent être reconsidérés expérimentalement. En réalité, notre technique nous a permis de constater avec certitude que les éléments étudiés par ces auteurs ne sont pas tous des réticulocytes, mais, en grande partie tout au moins, des hématies à granulations de Heinz.

Nous poursuivons actuellement, en collaboration avec A. Lambrechts, des expériences sur le comportement respectif des réticulocytes et des granulations de Heinz au cours de l'intoxication aiguë par la phénylhydrazine. Notre technique nous permet de procéder *simultanément*, sans la moindre possibilité de confu-

sion à la numération comparative de ces deux éléments sur un même frottis de sang.

Les granulations de Heinz sont bien visibles sur le frais, sans coloration, en chambre humide. Elles apparaissent admirablement sur fond noir. Il arrive qu'elles se détachent de la paroi globulaire et se retrouvent, en liberté, animées du mouvement brownien.

Remarques: a) la coloration au bleu de crésyl brillant combinée avec l'examen sur fond noir assure la distinction de la substance reticulo-filamenteuse et des granulations de Heinz, grâce à la coloration différente de ces éléments sur fond noir et sans recourir à des méthodes de coloration d'une précision et d'une régularité relatives.

b) Contrairement aux assertions de P. Dustin Jr. (10), le rouge neutre ne met pas les granulations de Heinz obtenues par la phénylhydrazine en évidence sur frottis desséchés; après coloration post-vitale, sur fond clair ou sur fond noir. La distinction de ces éléments d'avec les réticulocytes, colorables par cette méthode, est donc assurée.

c) La technique de coloration de Manson-Schwartz combinée avec l'examen sur fond noir ne montre pas les granulations de Heinz et ne colore pas les ponctuations basophiles (voir plus haut). La distinction de ces deux derniers éléments est donc également assurée.

Conclusions.

La technique d'examen sur fond noir de préparations colorées permet une identification facile, sûre et précise des différentes structures granuleuses des globules rouges. Elle permet d'éliminer de nombreuses confusions et erreurs commises jusqu'à présent. Cette technique repose sur une observation fondamentale, à savoir, que certains colorants manifestent sur fond noir des colorations électives et des métachromasies que l'examen classique sur fond clair ne met pas en évidence. Ce phénomène est certainement susceptible d'une application étendue en histologie. Nos techniques sont particulièrement favorables à l'étude des granulations et des fines structures. Il est nécessaire de faire preuve de discernement et de prudence.

Des éléments invisibles sur fond clair apparaissent sur fond noir, mais le contraire se présente également. C'est ainsi qu'il

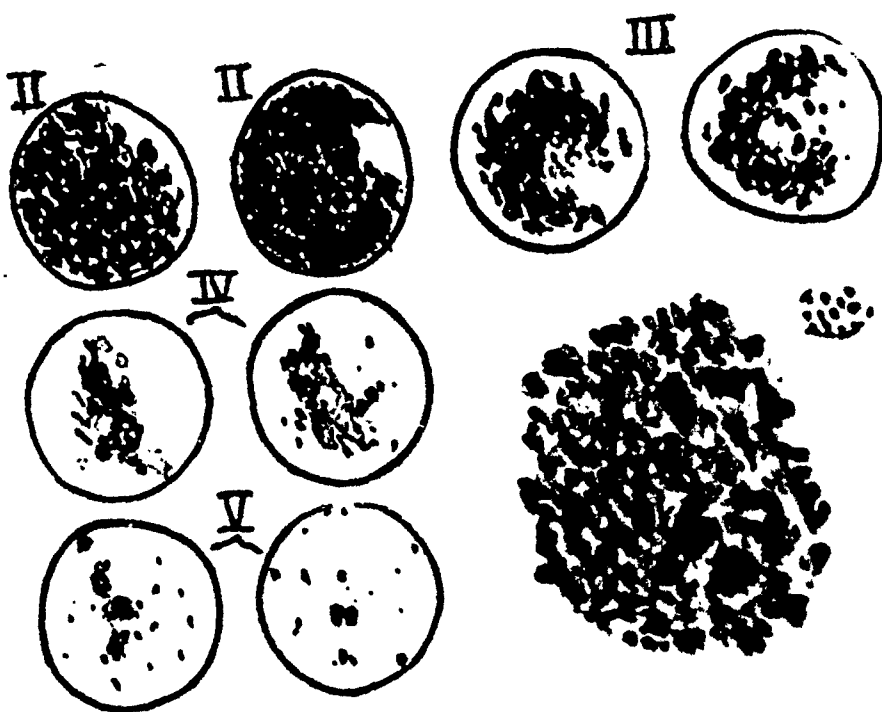
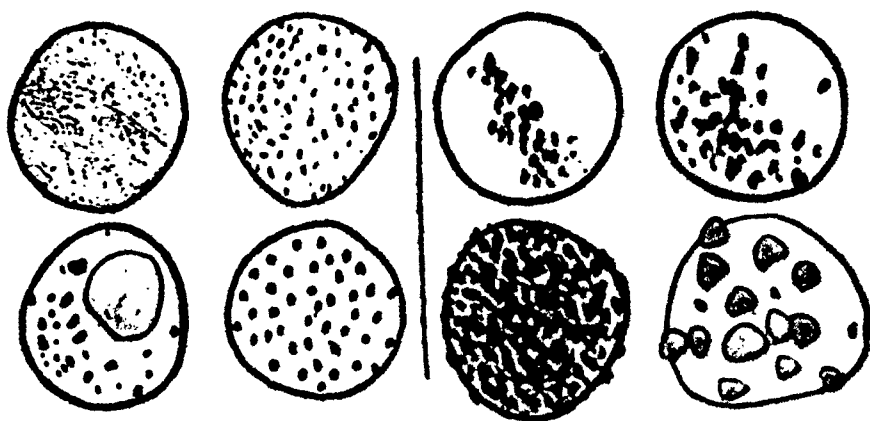


Fig. 2. Réticulocytes (Gr. II, III, IV, V); leucocyte, plaquette.



Ponctuations basophiles.

Granulations de Heinz.

Fig. 3.

est possible par l'observation sur fond noir en faisant disparaître certains éléments visibles sur fond clair, de mieux mettre en évidence des structures déterminées (voir plus haut: observation des granulations basophiles dans les normoblastes).

Divers auteurs ont appliqué la technique du microscope électronique à l'étude des hématies (Wolpers) (66) Jung (33). Dans l'état actuel de cette technique et pour certains buts, nos méthodes paraissent préférables. Lorsqu'il s'agit de la mise en évidence de fines granulations, elles lui sont supérieures, grâce à l'effet ultramicroscopique. La technique du microscope électronique est coûteuse, compliquée; elle réclame des manipulations plus complexes, source d'artefacts; inconvénient très grave, elle ne comporte pas la coloration spécifique des éléments; de plus, elle ne permet d'étudier que peu d'éléments à la fois; elle se prête donc mal à l'établissement de formules. Nos techniques, au contraire, sont simples et précises, rapides, peu coûteuses, peu fatigantes. Elles comportent peu de causes d'erreurs. Elles permettent l'identification précise des diverses structures par l'emploi de colorations spécifiques. Enfin, elles rendent possible d'examen rapide et direct d'un grand nombre d'éléments et l'établissement commode de formules sanguines précises.

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(From Med. Clin. Akad. Hospital, Upsala (Sweden). Chief: Prof. G. Bergmark).

Incipient myelomatosis or «essential» hyperglobulinemia with fibrinogenopenia — a new syndrome?

By

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The real nature of myelomatosis.

The title of this paper may at first seem somewhat surprising. The myeloma has of old had a reputation as a well defined clinical entity. With the aid of the typical changes on the X-ray film and guided by the examination of the cells from a sternal puncture the diagnosis should therefore be easy and there ought not to be found any serious diagnostical troubles. In the following I am going to give a description of two cases, who have several symptoms suggesting myelomatosis but also show decided differences. They are very much alike even as regards details in the chemistry of the blood proteins and it seems probable according to my opinion, that they suffer from the same malady. A third case very much resembles these two patients but also shows other signs, that do not fit in so well with the picture.

It might be possible to regard these patients as myeloma without myeloma i. e. the hypothesis may be propounded, that we have to do with a premyelomatous stage of the malady with only increase in serum globulins but not yet with the secondary deposition of myeloma tissue, plasma cells, in the bone marrow (Waldenström 1942). I have recently pointed out, that many facts in the

clinical picture of myeloma would be explained, if we assume that the malady were really a thesaurismosis. The primary change should be a deranged protein metabolism with secondary deposition of these pathological products, above all globulins, in the bone marrow. If this were true the disease ought not to be regarded as a true neoplasm but rather as an analogon of the so-called lipoidoses e. g. Mb. Gaucher or Schüller-Christian (Also the skeletal xanthomata were until quite recently regarded as tumours). The real cause of the disturbed protein metabolism is as yet unknown but some possible explanations will be discussed later.

Before I proceed to a discussion of the diagnosis etc. I shall give the case histories.

Case histories.

Case I. Farm labourer born in 1881. Nr. 794/42. Hereditarily nothing known (mother dead, father unknown). Previous maladies of no interest. In Aug. 1935 pains in the shoulders and left wrist. Admitted to the Medical Clinic on Aug. 19th (for hematological data see table 1).

From internal organs nothing of interest. Blood pressure 145/110. Left-sided hydrocele, the size of cocoanut. Left shoulder no swelling or redness but diffuse painfulness on pressure chiefly from the axilla. All movements painful. Other joints show nothing abnormal. No anemia, W. R. neg., no albuminuria. X-ray: Left shoulder: no skeletal changes, no calcified bursa. Heart: aortic configuration. Lungs: normal. No fever. Later several severe nose bleedings which caused some anemia. Dismissed on Nov. 22d «no cause of the increased S. R. has been found». — Ordered iron pills and active movements. After springtime 1936 no troubles from the joints but several, severe, nose bleedings. In the springtime 1941 3 weeks in the Otiatric Clinic for these complaints. On March 16th 1942 «influenza». Admitted on March 21st with typical signs of lobar pneumonia. Has had no severe bleedings lately. From status: Dyspnoic, slight edema of the feet, some cyanosis. Rather severe eczematous changes on the trunk. Slight on arms and legs. Typical signs of pneumonia sin. (X-ray) Heart nothing abnormal. Bloodpressure 180/115. High fever. Blood values: see table. The condition of the patient improved rapidly. Fractional test meal: Free HCl 55 after 0.2 g caffeine. Serumiron 56 γ %; non protein nitrogen 43 mg %. Meulengracht 2. Electrocardiogram: nothing pathological. From the hydrocele were drawn 2 litres of fluid. X-ray of cranium and pelvis: no signs of myeloma. W. R. neg. (slight auto-inhibition). The status of the patient was controlled several times. He usually showed some albuminuria, but no sediment or Bence-Jones proteinuria. Blood examination: see table. Has been able to work during the autumn but has had some severe nasal and gingival bleedings. No pains of any

kind. The patient was treated several times at the Medical Clinic with venesections, retransfusions of his own erythrocytes and transfusions. No real change in the status during the year but there were found a few enlarged lymph glands. One of these was excised. After this operation *bleeding from the wound for more than a fortnight*. Micr. picture: catarrhal changes in the sinuses with localised fibrous and hyaline induration. Some increase of plasma cells. No signs of tumor. Decidedly no myeloma (Gellerstedt).

In *Jan. 1943* new transfusion after severe epistaxis. Coagulation-time tested with glass-bead in a small test tube 11.5 min. (normal person 3.25 min.). Bleeding time 8.5 min. (normal person 2.5 min.). Meulengracht 4. The values for citric acid and phosphatase were normal. The patient was treated with transfusions of in all 900 cm³ and large venesections of a litre; the red corpuscles were allowed to sediment and were reinfused. After this treatment no bleedings.

Readmitted to the Surgical Clin. on *March 23rd 1943* for severe abdominal pains on the right side. From the status: severe meteorism and painfulness in the right upper abdominal quadrant. Leucocytosis of 14000 cells. In the sediment there were found some erythrocytes. The pains disappeared after a few days. The patient was treated with large transfusions of in all 1300 ml blood. In spite of this not more than 1.5 mill. Ery. Bloodpr. 115/75. Persistent slight albuminuria. No casts Bence Jones neg. Water tolerance test (1000 ml). In 2 hours 280 ml; lowest spec. grav. 1,012. 4 hours 405 ml, 1370 ml in 24 hours, with highest spec. grav. 1,021. Decreased from 65—63.3 kg in weight. Bloodpressure 115/75. Sternal puncture: no marrow could be aspired. On April 3rd suddenly pains in the right knee joint (probably bleeding). Ocular fundi show multiple bleedings round the papillae. X-ray of the skeleton only showed a periosteal thickening on the left femur.

A new water tolerance test was performed with 1500 ml instead of one liter. During the first 2 hours excretion of 310 ml in 4 hours 515 in 24 hours 1400. Spec. grav. 1,017—1,020. After several transfusions his status was considerably improved. He had no bleedings and felt much better. Dismissed with Hgb 30. Red. 1.6, White 7000 on May 27th.

The serum and blood of the patient showed many peculiarities which will be discussed and described in the text.

Case II. Farmer born in 1878. No 2394/1942. Hereditarily nothing of importance. In 1928 trauma against the left eye, with luxatio lentis et ablatio retinae. Since Aug. 1941 decreasing vision on the right eye. Treated in the Ophthalmiatric Clinic Aug. 9th—Aug. 16th 1941 for thrombosis venae centralis retinae dxt. Since then status quo. Is not able to read. Oct. 1941 bleedings from nose and gingiva. Treated with injections without any certain effect.

Admitted to the Med. Clin. on April 29th 1942. No dyspnoea, no fever, some bleedings from the gingiva. Some enlarged lymph glands in the fossae supraclaviculares and below the mandibula. Heart: nothing abnormal.

Blood pressure 120/85. No albuminuria. Urobilin: traces. Urobilinogen neg. X-ray: skeleton no signs of myeloma. W. R. neg. Meulengrath 2. Citric acid 31.4 γ /ml. Phosphatase 3.6 Ph. units. Readmitted several times during the following months for bleedings. Blood values see table. Treated with large transfusions; bleedings very much improved. Feels better than for several years. In Oct. and Dec. 1942 and Febr. 1943 readmitted for transfusions. X-ray skeleton several times, no signs of myeloma. Serum calcium normal, citric acid very high (79.7 γ /ml.) Phosphatase low. Meulengrath 4.

Readmitted on April 7th 1943 for transfusions. Has been quite well for about a month after the last transfusions. During this time no bleedings from the nose or mouth but these have relapsed of late. Has felt very tired and dyspnoic. No edema, no pains of any kind. On admittance gingival and nasal bleedings. Lymph glands as before. Heart nothing abnormal, blood-pressure 125/95. No albuminuria. Hgb 36 %, Ery 2.1 mill. Leucocytes 9500. Platelets 120000. Prothrombin index 103. F-gel 10 sec. Retic. 12% Coagulation time (Howell-Gram) 10 min. A sternal puncture was attempted but it was impossible to aspire any fluid through the needle. There was continuous bleeding from the wound for more than 12 hours. Water tolerance test. 1000 ml. After 2 hours 545 ml, 4 hours 790 ml., 24 hours 1800. Spec. grav. varied between 1.009 and 1.020. Citric acid in the serum 76.8 γ /ml. Phosphatase 2 Ph. un.

Readmitted on May 27th 1943. Has felt better but never been free from bleedings which have increased considerably during the last days. The mouth has been filled with blood. Status as before. Blood pressure 105/75. Hgb 20 %, Ery 1.3, No albuminuria. Platelets 115000. After a transfusion considerable improvement. Hgb 30 Ery 1.6. White 6700. Dismissed on June 10th.

Case III. Farmer born in 1880. No. 1095/1941. Hereditarily nothing of importance, no previous maladies. In 1940 some coughing and anemia. Treated with injections. Admitted on April 16th 1941 for fatigue and dyspnoea. No pains, no loss of weight, no fever. Some lymph glands the size of a bean on the neck, in the axillae and inguina. Internal organs otherwise normal. Blood pressure 120/70. No albuminuria or urobilinuria. Free HCl 75 after 0.2 caff. A lymph gland was excised. Microscopical diagnosis: localized proliferation of the reticulum but without any signs of granuloma or tumor. Partial reticulosis (Gellerstedt). W. R. neg. No autoinhibition. Blood values see table.

The status of the patient has been followed several (7) times in the Outpat. Dep. The Hgb was varied between 56 and 38 %. The Ery 3.8 to 2.0 mill. Serum iron low 25 γ %. Persistent slight albuminuria. No Bence Jones protein. Sed: nothing pathological. The skeleton was x-rayed several times but nothing pathological was found except a spondylitis deformans.

Readmitted on March 18th 1943 for increasing fatigue. Otherwise no special symptoms, no pains. Moderately enlarged lymph glands as before. Blood pressure 120/70. X-ray: Skull, vertebral column, no signs of mye-

Table 1.
Chemical and hematological data.

Date	S. R. mm/hr.	Hgb %	Ery mill.	Leuco- sytes 1000	Plate- lets 1000	For- mol- gel	Total prot. %	Alb. %	Glob. %	F-gen %	Albu- min- uria
Case I.											
1935											
5/10	135	80	3.5	5							
20/11	132	85	3.2	8							
1936											
30/1	117										
1941											
10/6	145										
1942											
23/2	130	55	2.9	9		1'					
9/4	145	50	2.9	6			8.4	2.3	6.1		(+)
19/10	150	40	2.0	7		10''					
18/11	150	45	2.5	8	115	15''	10.8	2.5	8.2	0.09	(+)
1943											
28/1	165	35	1.4	7	120	15''	10.2	2.2	8.0	0.25	
23/3	176	25	1.1	14	73		10.0	2.3	7.7	0.39	+
25/5	157	30	1.6	7		50''	6.6	2.0	4.6	0.67	—
Case II.											
1942											
30/4	150	45	2.1	10	85	14''					—
5/5	140	40	2.0			10''	12.4	2.2	10.2		—
9/6	140	40	1.8		150	15''					
16/7	153	40	2.1			7''	11.4	2.1	9.4	0.14	—
13/8	152	43	2.2		85	14''					—
19/10	156	40	2.1	8	82	11''					
11/12	160	40	2.0	7	91		12.8	1.9	10.8	0.07	—
1943											
9/2	160	30	1.4	4	150	9''					—
9/4		35	2.1	9.5	120		11.5	2.0	9.5	0.2	—
25/5	160	26	1.9	0		10''	12.4	2.2	10.3		—
Case III.											
1941											
16/4	140	56	3.1	9	440						—
23/4	120	56	3.5	11		15''					—
3/6	140	56	3.5			7'	10.6	3.3	7.3		
25/8	140	51	2.7	10							
19/12		43	2.7	13							+
1942											
5/2		43	2.2	13							+
1/4	156	38	2.1	10		2'					
6/6	151	47	2.7	10	457	10'					
1943											
19/3	160	25	1.3	10	353	24'	7.7	1.8	5.9	0.6	+
25/5		22	1.7	7	230	20'	6.4	0.75	5.7	0.7	++

loma. Bleeding time 3.5' Coagulation time (Howell-Gram) 7' Albuminuria 1—2.5 ‰. Non protein N. 30 mg %. Kongo red test: after one hour very slight blue colour of plasma after addition of HCl. No coloration in the urine with HCl.

Readmitted on May 27th. Very feeble. Bad appetite. Edema of the legs, abdominal wall and hands. No bleedings. Heart normal, blood pressure 80/35. Liver enlarged. Albuminuria 2.5—4 ‰, but no Bence Jones protein. Capillary resistance: no bleedings after 50 min. pressure for 15 min. or 100 mm for 15 min. X-ray of vertebral column, pelvis and cranium no signs of myeloma.

The general status was very poor. The patient had no appetite at all. His edema increased considerably (very severe hypo-albuminemia!) in spite of blood transfusions. After a period of diarrhoea the patient died on June 18th.

Differential counts from blood and sternal punctures from Cases I—III.

Case I.

Blood.

	Neutroph.	Eo	Baso	Lymphoc.	Monoc.	Normobl.	Myeloc.	Plasmacells
21/4	42	50.6	6.5	—	37	6		
19/10	»	55	1	—	36	8	2/200	
17/11	»	51.5	0.5	—	45.5	2.5	3/200	
27/3	43	56	2	—	30	8.5	2 %	1.5 %

Bone marrow.

17/11 42. Myeloblasts 0.6 % Neutrophilic myelocytes 2.0 Neutroph. leucoc. 23.2 Monocytes 2.6 «Lymphocytes» 70.2 Plasma cells 0.4 Normoblasts 1.0.

27/3 43. N. Myelocytes 2.0 % Eosinophilic m. c. 0.2. Neutroph. leucoc. 28. Monocytes 2.0. «Lymphocytes» 64. Normoblasts 3.2. Plasmacells 0.4. Rare erythrocytes show polychromasia and basophilic stippling. Slight anisocytosis. Very few typical plasma cells. No cells suspect for myelomatosis.

Case II.

Blood.

	Neutro.	Eo.	Baso.	Lympho.	Mono.	Normoblasts.
10/6	42	40	2	—	54	8
23/7	44	1	—	50	3.5	2/200
19/10	60	0.5	0.5	35	4	1/200
15/2	43	49	—	0.5	44.5	4

Bone Marrow.

10/6 42. Neutrophilic myelocyte 0.6. Neutrophilic leucocytes 1.8 Normoblasts 0.4 «Lymphocytes» 93.2. Plasma cells 4 %.

The lymphocytoid cells were mostly spindle shaped with protoplasmatic bodies often separated from the mother cells, sometimes vacuolized.

Case III.

Blood.

Neutro.	Eo	Baso.	Lympho.	Mono.	Normoblasts.
3/6 41 54	1	0.5	34	10.5	
19/12 41 39	1.5	0.5	31.5	8	
1/2 42 40	2.5	—	51	6.5	
6/6 42 58	0.5	—	35	6.5	1/200
20/3 43 57	1.5	—	30.5	9	2/200

Bone marrow.

16/4 41. Myeloblasts 0.6. Promyelocytes 0.8 Neutrophilic m. c. 13.8 Eo. m. c. 0.4. Neutrophilic meta m. c. 0.6 N.phil. leucoc. 33.8. Lymphocytes 14.4 Monocytes 2.0. Plasmacells 3.4. Normoblasts 20.6.

1/2 42. Very cellular marrow. Plasma cells in groups very rare. Spindle-shaped »lymphocytes» are to be seen.

M. bl. 0.2 %. N. ph. m. c. 16.2. Eo. m. c. 0.6. N. meta m. c. 20.2. N. l. c. 29.0. Eo l. c. 0.6. Monoc. 0.6. Lymphoc. 13.6. Plasma cells 1.6. Normoblasts 16.8.

20/3 43 M. bl. 0.2 % N. m. c. 2.4. Eo m. c. 0.2. N. Metam. 3.0. Neutrophils 17.0 Eo l. 0.6. Baso l. 0.2. Monocytes 1.4. »Lymphocytes» 69.4. Plasma cells 1.6. Normoblasts 3.4.

Very frequent darkly basophilic protoplasmatic bodies, partly vacuolized, partly resembling polychromatic erythrocytes. It is sometimes clearly to be seen, that they originate from the lymphocytoid cells. Such bodies are not to be found in the blood. Very numerous naked nuclei.

Discussion of the diagnosis.

The *clinical picture* in the first two cases is very similar and this seems to indicate that they suffer from the same malady. An elderly man comes to the doctor because of symptoms, that are probably caused by an *anemia* with a tendency to bleedings. At the ordinary clinical examination we find a generalized slight enlargement of the lymph glands with considerable anemia. This is normochromic or slightly hyperchromic, without signs of hemolysis (bilirubinemia or urobilinuria) or increased regeneration (reticulocytosis). A certain leucocytosis was found in Case II. His blood also showed mononuclear cells of a type that is difficult to determine. The *bone marrow* has the same appearance in both cases with *chiefly lymphocytoid* cells. On repeated examinations plasma cells were only found in

normal percentages or at the upper limit of the normal. The white blood cells show nothing, that indicates a leukemia. The patients have been followed for a long time (one for more than seven years, the other for two years without any rapid changes in the status). Among the clinical features the excessive *sedimentation of the erythrocytes* is one of the most striking. A detailed analyses shows, that it is caused by a very marked *increase* in the *globulin* content of the *serum*. *The content of fibrinogen* however is *pathologically low*. These facts make it possible to characterize the cases and place them in a special group. Is it possible to correlate them with other wellknown maladies?

A diagnosis that has always been the cause of much discussion is chronic aleukemic lymphadenosis. As far as this condition later develops into a manifest lymphatic leukemia the diagnosis aleukemic leukemia seems to be legitimate. Sternal puncture may lead us to suspect this malady in cases without visible enlargement of the glands or typical blood picture. Such cases with a primary proliferation of lymphoid cells in the bone marrow later followed by a typical leukemia are of very great importance. They are very rare (cf the work of Hynes 1940 where no case developed a real leukemia). Very often there is really an aplastic anemia present which later develops into a final acute leukemia.

This diagnosis: panhemocytopenia with or without myeloblastic proliferation (so-called myeloblastic leukemia) might also be discussed because of the atypical bone marrow picture.

The longdrawn clinical course however speaks against it, nor was it possible to find any typical pathological myeloblasts among the small mononuclear cells found at sternal punctures.

Is marked increase in serum globulins a sign, that has been described in leukemia? Snapper publishes a rather extensive study concerning this problem and he points out, that hyperproteinemia speaks against the diagnosis uncomplicated leukemia. In rare instances S. found border — line values. Out of 15 cases one showed a globulin value of 3.3 % (with 1.0 % euglobulin). It was an instance of myelosis chronica. In a case of chronic lymphatic leukemia the value was 3.0 %. Low values for the fibrinogen have not been published as far as I know.

Isolated cases with marked hyperglobulinemia in the literature are reported to have shown signs of leukemia at the postmortem.

Gross has published a patient, whose malady was interpreted as aleukemic myelosis with a protein content of 14.8 %. Keilhack in a similar case but without post mortem found 8.6 % globulin of which 75.6 % was euglobulin. The patient had 9000 leucocytes in the blood; of these some were unripe. Thrombocytopenia. No X-ray examination of the skeleton. Bürkel places his case, that is often quoted, among the atypical myelomata and it is evident that also the anatomical diagnosis may be very difficult and uncertain. Some of the above-mentioned patients may possibly have suffered from the same malady as my two patients.

Snapper discusses the possibility of a simple combination of the two independent maladies: myeloma and leukemia in a patient he was able to observe. In my cases the long-drawn course (up to 7 years without the appearance of signs of real leukemia), the histological picture of the bone marrow, the severe fibrinogenopenia, the remarkable increase in serum globulins together with the absence of the common clinical signs of leukemia speak against this latter diagnosis.

In none of my patients was there any pains indicating a skeletal affection but in some cases recently published, who showed a widespread myelomatosis at the section, there were never any pains (Cases 1 and XII, Waldenström, 1942). X-ray pictures have been taken of the skeleton at different stages of the malady but no signs of myeloma have been found. As a matter of fact this does not prove that there is not present the form of the malady, that we call general myelomatosis. The result of the sternal punctures seems more important and the bone marrow has therefore been examined on several occasions. Both in Case 1 and Case II there was found a picture that is definitely pathological. According to the experiences put down in literature and also with regard to my own of 10 cases with myeloma, the picture does not in any way resemble what we find in this malady. There is not any decided increase in plasma = myeloma cells; the pathological cells are all very small with no characteristic histological details.

The third patient differs from the others in certain respects. His platelet counts for instance are constantly elevated not decreased as with the other patients, the formol-gel reaction is not so rapid, even if the value for the globulin is very high, he does not suffer from any bleedings and shows no fibrinogenopenia, he has a

more marked leucocytosis and lately he has developed a massive albuminuria indicating a «nephrosis». At the present stage it is not easy to judge if these differences are more important than the resemblances. At the last sternal puncture plasma cells in small clusters have been observed. This may possibly speak in favour of the diagnosis multiple myeloma (cf. Waldenström, 1942).

The question is near at hand if there are no exactly similar cases published in the literature. I have tried to go through all papers, that may possibly have some bearing upon this question and I have found a few instances with a very similar clinical picture. One was published by Bing and Plum (later also by Gormsen 1942). This patient was also an elderly man (65 years). For 3 years troubles with nosebleedings and melaena. After 2 years also impaired vision. He was examined in a hospital where the diagnosis retinitis haemorrhagica was made. Hyperchromic anemia (45 % 2.1 mill.) 6900 leucocytes. The diagnosis lymphatic leucemia was made and the patient has since been regarded as an instance of this malady. He had severe hyperglobulinemia (8—8.9 %) strong formol-gel reaction (2') and a S. R. of 160/hr. A later differential count showed 50 % lymphocytes; 43 % neutrophils and 7 % monocytes with a total count of 5000. Serum calcium 11.4 mg%. Bleeding time 10 min. A year after the first status the patient showed no enlargement of the lymph glands. Liver and spleen normal. X-ray of the lungs showed stasis? Bence Jones in the urine positive. Free HCl in the gastric juice. Blood count as before. On X-ray no signs of myeloma. Sternal puncture: 58 % lymphocytes but only 1 % plasma cells. The possibility of a myeloma was therefore discarded. It was impossible to follow the patient any further. The diagnosis lymphatic leucemia as a matter of fact seems very uncertain and it is at present impossible to ascertain if the malady was rather an incipient myeloma or the same as in my patients. The resemblances in the clinical picture are marked but there were no determinations of the fibrinogen (of the bleedings) or any more detailed investigation of the serum proteins.

Among Bing's cases there is another history (1940 Case 69) of a man 75 years old. He showed extensive moderate enlargement of the lymphatic glands. The leucocytes terminally rose to 55000 with about 50 % atypical lymphoblasts. Otherwise neutrophilic leucocytes. Formol-gel 2.5. Globulins 6.9 % High fever. The post

mortem gave no certain diagnosis but showed generalized enlargement of the lymph glands. A para-aortal gland was examined microscopically. It showed complete loss of structure with a generalized proliferation of rather large mononuclear cells somewhat resembling plasma cells. In the red bone marrow there were found numerous large and small non-granulated mononuclear cells not resembling lymphocytes. Some plasma cells. The case was interpreted as a septicemia with a leucemoid reaction, also the diagnosis leucemia was discussed. The resemblance with my patients seems obvious. The label reticulosis may also be used but it hardly furthers the understanding of the process.

In 1938 Rohr discusses the problem hyperglobulinemia and reticulum cells.

von Marsovszky (1940) has published a case of a similar type. A man of 50 showed severe bleedings from the nose and gums in 1939. He suffered from some anemia (58 % 3.2 mill.) Platelets 150000 S. R. 135 mm. The erythrocytes could not be counted in Hayems solution but only in normal saline. He had large retinal hemorrhages. Skeleton: X-ray normal, sternal puncture nothing pathological. Bence Jones neg. Total protein in the serum 12.2—16 %. Albumin 1.4—0.7! Fibrinogen 0.6—0.7 %.

The post mortem showed nothing definitely pathological. v. M. published the observation under the title: Hyperproteinemia causa incerta. It is obvious, that the clinical picture in all essential respects is identical with my cases, only the value for fibrinogen was increased. It is to be noted however, that the fibrinogenopenia was no constant finding in my cases either.

In 1940 Malmros published three cases of hyperglobulinemia, where it was impossible to find any cause of this symptom. We have been able to observe a number of similar patients in the Medical Clinic of Upsala these last years. Some of them are now published in *Nordisk Medicin* (1943). They are decidedly not instances of the same pathological process as was present in Case I and II.

Fanconi in 1941 publishes an instance of »dysproteinemia» in a luetic infant. The blood was highly viscous, did not coagulate but there was coagulation of the plasma at 56°.

Some clinical and chemical observations.

One of the most striking properties of the sera from Cases I and II was their very *high viscosity* already at room temperature. I have therefore tried to investigate this property more closely especially as the viscosity of the serum in hyperglobulinemia has been very little studied. From later years there are only a few determinations in cases of myeloma by Albers and Magnus Levy but no attempts at a systematic analysis have been made.

For the measurements I have used a slightly modified Ostwald's viscosimeter, an instrument whose simplicity makes it well suited also for clinical work. The only real draw-back with the original instrument is the difficulty to tell beforehand, what will be the correct time to read the instrument and this leads to a series of unnecessary observations in order not to miss the right moment when the meniscus passes the lower limit. I have therefore constructed a modification of the original instrument, where it is possible first to determine 1/10 of the total time. This is usually done quickly. A clock is then started that gives a signal when the instrument ought to be observed again. With the aid of this modified instrument it seems, as if viscosimetry would be a simple and useful clinical method.

All determinations are performed in a waterbath and the temperature is corrected with automatic thermoregulator, when the difference against room temperature is great. The fall-time for a number of sera with increased globulin content was determined at different temperatures and with different instruments. The determinations with viscosimeters having varying dimensions of the capillaries gave no real differences even in sera with very high viscosity and the results will not be discussed here.

Determinations at different temperatures were made in order to find out, if the very marked temperature dependance of viscosity found in the serum from Case I might be observed also in the sera from other patients. Measurements were therefore made at every 4th grade from 9°—39° C on a number of sera (see fig. 1). In others determinations were only made at a few definite temperatures (usually 13° and 37°). The temperature 17° has also been used in a number of instances, as it corresponds well with ordinary room temperature. Under such circumstances there are certain impor-

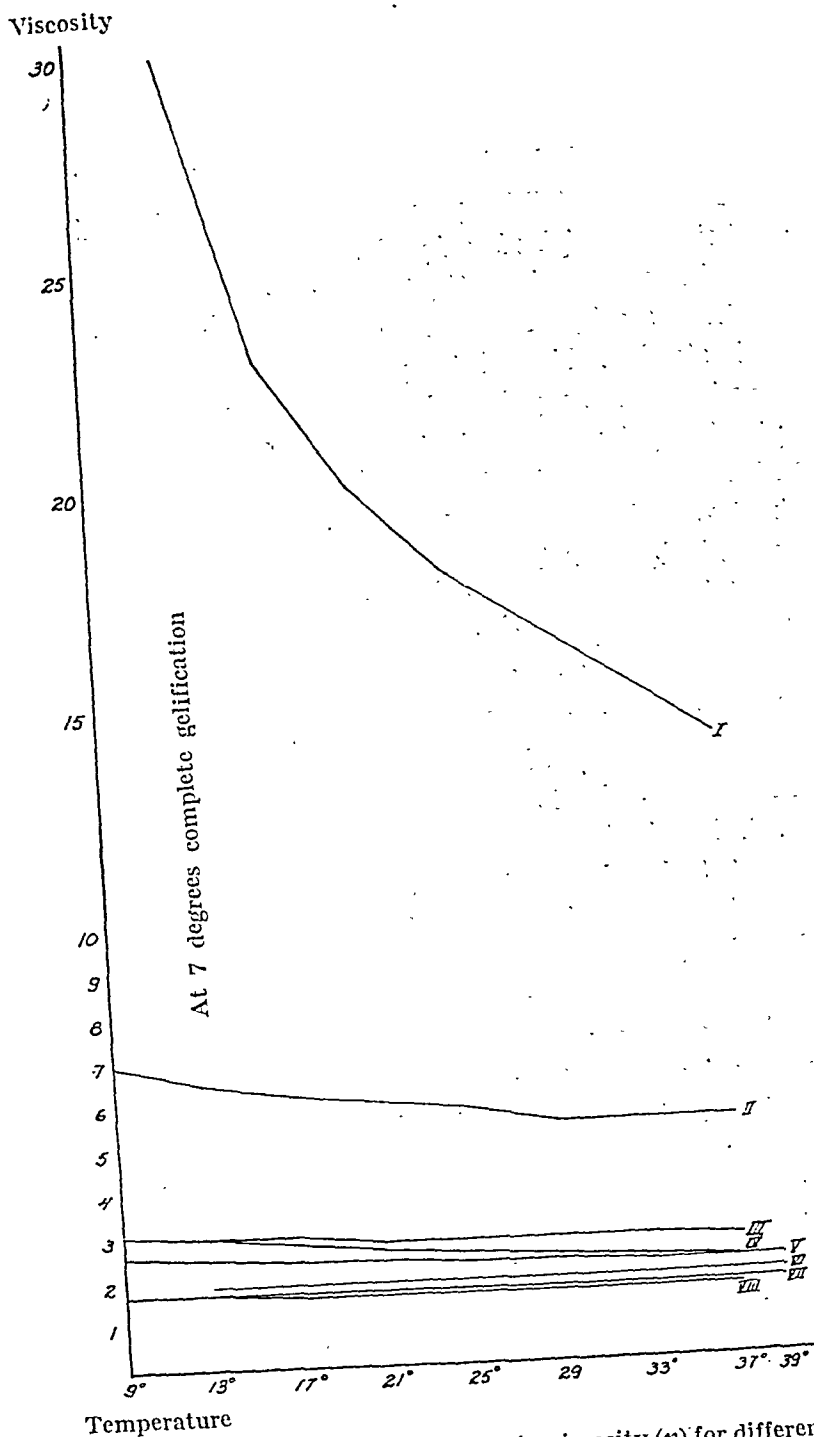


Fig. 1. The curves show the very great difference in viscosity (η) for different sera. I represents the serum from case II. It is seen that the viscosity is very high at all temperatures but also that the influence of the temperature in this case is much more marked than in any other. Curve II shows the very gradual increase in the viscosity of the serum from case I with falling temperature. At 7° complete gelification however. Curve III shows the serum from a case of myeloma with high globulin content but no very marked viscosity or increase with falling temperature. Curve IV shows that the serum from case I has still a very marked increasing viscosity with falling temperature even after dilution 1:1 with saline. Curves V and VI show the very slight increasing viscosity with falling temperature in two other instances of hyperglobulinemia. A normal serum VII has absolutely the same form as a curve for water. Curve VIII gives the subnormal values for a serum dilution 1:3 from case II but with still marked increase at low temperature.

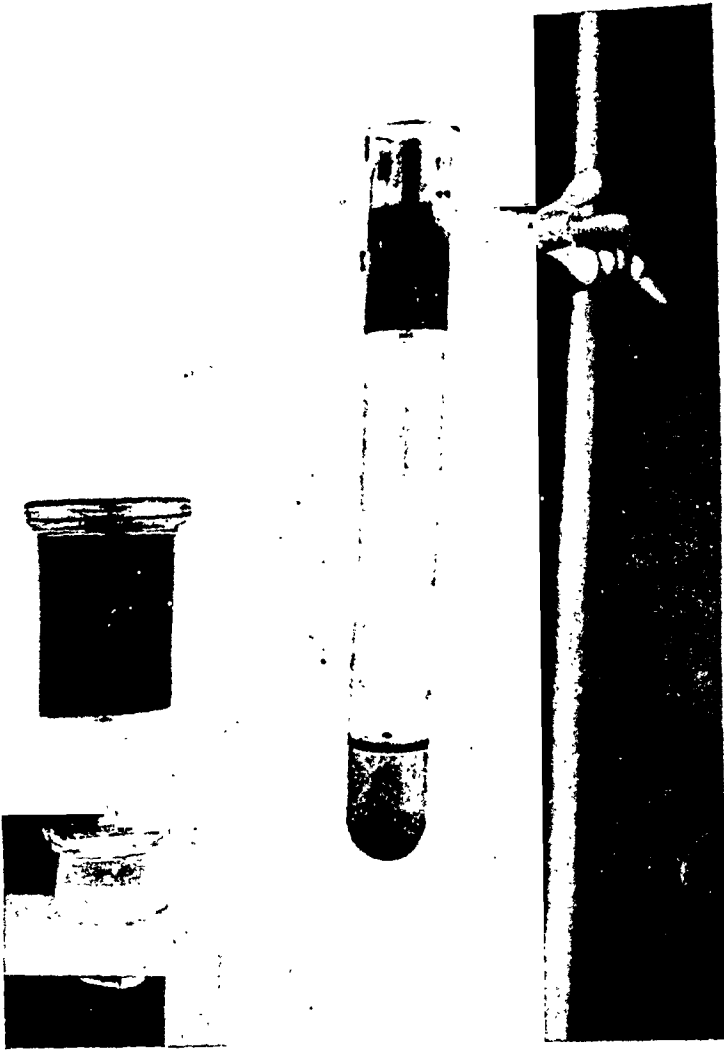


Fig. 2. The stoppered flask that is put upside down contains serum from case 1 that has been kept in a refrigerator at $+ 5^{\circ} \text{C}$. The serum is gelified and opaque. In the test tube a sample of the same serum kept at room temperature. It is quite limpid (but viscous) and translucent.

tant facts to be observed. The term relative viscosity (η) is used to denote the relation of the fall-time to that of water or normal saline; the very slight influence of specific grav. is disregarded. For many sera it is quite constant in the temperature range 9° — 37° . They behave in other words just like water and there is only a slight increase in viscosity with sinking temperature. Some of the sera with high globulin content behave in quite another manner. In one patient with a serum globulin value of 5.7 % the

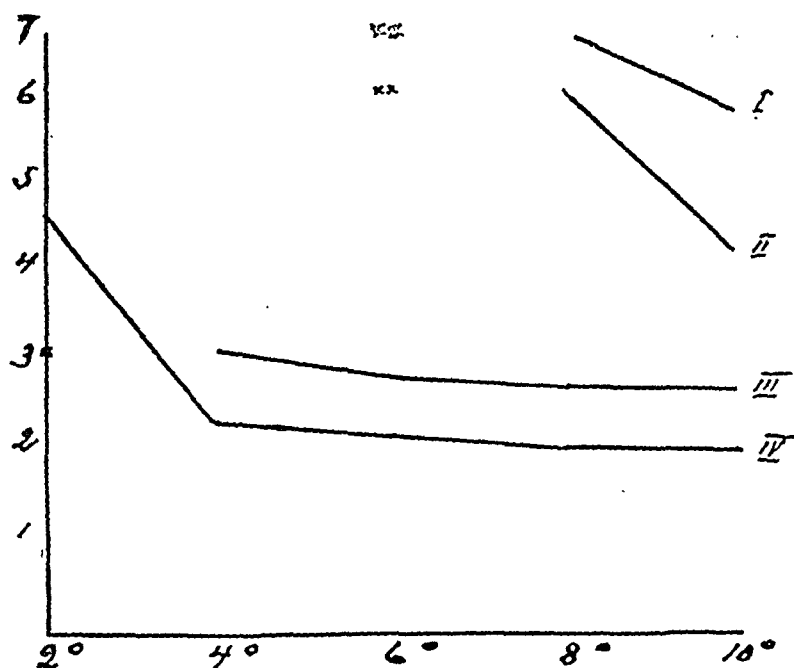


Fig. 3. The influence of dilution on gelification of the serum from case II at low temperature.

I Undiluted serum ++ Gelification at 6°

II 2.5 ml serum + } ++ " " "

0.5 ml saline

III 2 ml serum + 1 ml saline + Gel at 2°

IV 1.5 " 1.5 " + " " "

In dilution III there is no gelification above 2°. In IV there is no gelification whatever.

η at 13° was 2.7, at 17° = 2.6, at 37° = 2.4. In cases I and II the differences were still larger.

A patient, who suffers from myeloma and shows a serum globulin of 10.2 % showed a relative viscosity at 17° of 3.7. In another patient with 8.7 % globulin it was as high as 6.2 but in a case with 10.8 % globulin it was only 2.3. Also a dilution of the most viscous sera to half the initial globulin value gives a much higher viscosity than was found in other sera with the same original globulin content.

These relatively large differences are not merely a result of a very high globulin content as another patient with a still higher globulin value (5.9 %) and the same viscosity at 13° (2.7) only showed 2.6 at 37°. The relation of viscosity to temperature is

thus different in different patients and is probably to be regarded as a result of certain qualitative differences in the globulin and not as a function of the globulin content.

An extremely high viscosity in relation to the globulin content seems to be characteristic for patients with what I call essential hyperglobulinemia but it has also been observed in two instances with myeloma and in one case of polyarthrititis with sepsis. In order to get an expression for the temperature dependance of the viscosity I have calculated the relation between relative viscosity at 13° and at 37° and multiplied the value by 100. This index seems to give a rather good expression of the temperature variability of viscosity. In normal sera it lies at 100 or very near this value. The highest value I have found is 211 i. e. the viscosity is more than doubled from 37°—13°.

The variability of the *fibrinogen viscosity* with temperature was also determined in 12 plasmata from different patients with a high sedimentation rate. It is well known, that this protein has a high viscosity, and it is rather surprising, that the easily determined specific viscosity has not been used for qualitative or quantitative studies. The investigations of later years above all with the ultracentrifuge have shown, that fibrinogen after precipitation and resolution is easily denatured. I have therefore made some experiments with plasma and serum from the same patient. A man with a practically normal serum viscosity, who suffered from chronic polyarthrititis, had a very high plasma viscosity and a short plasma formol-gel time. Formol-gel neg. in the serum. The viscosity of the plasma showed a very marked dependance on the temperature with a value of 120 comparable to the serum globulins in case I.

In 11 cases the plasma viscosity was practically the same at 13° and at 37°, when the serum viscosity was normal, and the serum-formol-gel was neg. It may therefore be assumed, that fibrinogen usually does not show a very marked influence from falling temperature on its viscosity.

The serum from one of the here published patients (Case I) showed a very curious phenomenon. His serum and plasma were at room temperature highly viscous but not to such a degree as the serum from Case II (see fig. 1). If a sample is left in the ice chest at + 4—5° it develops into a jelly, that gets white and opaque (see fig. 2). At higher temperatures it is possible to «thaw» it up again.

The sample then »melts» peripherically leaving a solid centre. None of the many sera with hyperglobulinemia that I have been able to examine showed anything similar and I have not found the phenomenon mentioned in literature. With the aid of the viscosimeter it has been possible to examine the problem more in detail. It was found, that the sample had not become solid at 9° but at 7° it did not pass through the capillary. The serum was left in the apparatus over night at a temperature of 17° but this was not enough to lower the viscosity to such an extent, that more than a few drops passed the capillary. When warmed to 37° the serum again became liquid.

The influence of dilution was tested in the following way. (see Fig. 3). The nondiluted serum gelified at 6°. Also slightly diluted it behaved in the same way. In a dilution 1 part saline + 2 parts serum there was a measurable viscosity at 6° and 4° but at 2° there was complete gelification. Dilution 1.5 + 1.5 gave a high viscosity but no jelly at 2°. An increase in the number of water molecules present lowers the temperature for gelification. It is thus obvious, that the temperature limit is not dependent on any sudden change in the composition of the protein at a definite temperature but rather on its concentration. According to my opinion gelification is seen, when all water molecules of the solution are bound to the protein. I. e. there is no more free solvent present.

As it might possibly be suspected, that the high viscosity were caused by some mucin substance, even if this was not very probable, the serum was treated with a highly concentrated mucinase, that I obtained through the courtesy of Doctors L. Hahn and B. Skanse. The viscosity was not altered by the influence of this enzyme.

What is the real cause of a high viscosity? If it is allowed to make a generalization it would perhaps be suitable to say that it is caused by a thronging of the molecules in a fluid either because they are very long-drawn, thread-like or because there is such a high concentration of the dissolved molecules or such a tendency for them to bind water molecules that much of the water is fixed. Threadlike molecules and molecules with a strong tendency to hydratization are biologically the most important causes of a high viscosity. There are e. g. sera containing large amounts of globulins with a very high hydratization. If the concentration of these

molecules is high enough there develops a gel — all the water molecules are bound and there is no longer a free solution (cf. Case I).

In the two cases published here (I and II) there was a very high content of euglobulin in the serum that may be precipitated by a very simple procedure (dilution with H_2O or dialysis against water). The precipitate that is formed in this manner does not resemble an ordinary dry protein. It is a slimy translucent jelly, insoluble in water but easily soluble in for instance normal saline. It is obvious that the high viscosity is caused by this substance which in itself gives highly viscous solutions. The serum after precipitation of the euglobulin with dialysis is no longer very thick.

It is seen from Table I that there is no real parallellism between the values obtained from chemical or electrophoretical analysis of the globulin and e. g. viscosity or formol-gel time. Also the discrepancy between the formol-gel time and the viscosity is obvious. The five sera that showed the highest viscosity had formol-gel values varying between 24', 3'7", 50", 5'55" and 10". Among the sera with the shortest formol-gel time the viscosity was 3.7, 2.3, 6.2 and 20. What may be the cause of this?

Partly it may be explained by assuming a different chemical structure in the different globulins. It is obvious that the chemical structure of the very hydrophilic euglobulins in case I and II ought to differ greatly from other globulins. On the other hand the viscosity in Case D.-B. is also very high and lies between the values for Cases I—III even if he has no euglobulin increase and shows a rather long formol-gel time. It is probable that this last mentioned fact is caused by a low concentration of the dialyzable factor necessary for gelification with formaline. (Waldenström 1943). It is also possible, that the surprisingly low time in Case W. depends upon a milieu that is optimal for the reaction. When we know about the importance of low-molecular factors for the rapid formation of a gel such atypical instances, and also Jersild's patient with severe hyperglobulinemia and neg. formol-gel are less difficult to understand.

Another question seems to be of importance. Is it possible with simple means and without ultracentrifugation to get an idea about the molecular weight of a special pathological protein? This is obviously not yet the case. I have tried to find out if it were possible

Table 2.

The relation of relative viscosity and temperature for some sera with high globulin content.

Name Diagn. ¹	K. J. J. 1:3 Ess.	A. 1:3 Ess.	S. E. Ess.	A. P. Ess.	J. W. Prem.	E. K. Cirrh.	G. H. Sar- coid	L. F. Lupus cryt.	T. A. Endo- card.	I. B. M.	K. J. J. 1:1 Ess.	A. A. Case III Ess?	B. A. M.	O. Chron arthr.	A. 1:1 Ess.	K. J. J. Case I Ess.	D. B. M.	A. Case II Ess.
Glob. % Curve			4.5 VI	4.3	6.1 V	5.9	5.3	5.9	5.7	7.5 III	4.3 IV	5.9	10.2	7.3	5.4	8.7 II	7.8	10.8 I
9°		VII 1.7			2.6				2.8	3.1			4.1			7.0 ²		
13°	1.6	1.7	1.9		2.5	2.6		2.7	2.7	3.0	3.0	3.0	3.8	4.5	3.1	6.5		30.0
17°		1.7	1.9	2.1	2.4		2.4		2.6	3.0	2.8	3.4	3.7			6.2	6.7	23.0
21°	1.6	1.7	1.9		2.4				2.6	2.8	2.6		3.5			6.0		20.0
25°		1.7	1.9		2.3				2.5	2.8	2.5		3.4			5.8		18.0
29°	1.6	1.7	1.9		2.3				2.5	2.8	2.4		3.4			5.4		16.7
33°		1.7	1.9		2.2				2.4	2.8	2.3		3.3			5.4		15.4
37°	1.6	1.7	1.9		2.2	2.4		2.6	2.4	2.7	2.2	2.5	3.2	3.0	2.5	5.4		14.1
39°		1.7	1.9		2.2													
100 × η_{13} η_{37}		106			113	108		104	112	110	136	120	118	150	124	120		211

¹ Ess. = Essential hyperglobulinemia

Prem. = »Premyeloma»

M. = Myeloma

² = gelification at 7°

from determinations of the relative viscosity of the sera from Cases I and II to find out the shape of the molecules. This was not possible. The globulin component with a very high molecular weight that is present in these cases certainly gives the serum its high viscosity but in another highly viscous serum from a patient with myeloma (D.-B.) there was no component with such big molecules and on the other hand the serum of Case III. contained a large molecule but showed no very high viscosity.

At present it is necessary to collect more data about cases with abnormal serum globulins before we are allowed to state anything definite about the relative importance of different methods for the investigation of these substances.

Knowing the importance of the serum proteins for the *water transport* in the blood we may assume that a water tolerance test on these patients should give an abnormal result. In case III there was present a severe hypalbuminemia and consequent edema. No water tolerance test was therefore performed. In case II the result of the test was not extremely abnormal (2 hours 545 ml, 4 hours 790, 24 hours 1800, spec. grav. 1.009—1.020). In case I however there was a severe disturbance. After 1500 ml an excretion of only 310 ml in 2 hours, 515 in 4 hours, 1400 in 24 hours, spec. grav. varied 1.017—1.020. No hypertension, no casts but slight albuminuria.

It seems very striking that an increase of the specific viscosity of the serum to more than 10 times the normal does not cause any serious disturbance of the circulation and edema does not appear, when the albumin value is not very low (as in Case III). Determinations of the colloidal osmotic pressure have not been performed.

As the previous experiments have shown, that viscosity and total protein or globulin content of the serum do not run parallel it would seem possible, that some special globulin fraction caused the high viscosity. The globulin has of old been divided into two fractions: pseudoglobulin and euglobulin. They have different solubility in high electrolyte concentrations and the euglobulin is most easily salted out. According to Howe euglobulins are those proteins, that are precipitated by a concentration of 13.5 % Na_2SO_4 at 37°. The next fraction, precipitated at 17.4 % he called pseudoglobulin I and the last, needing 21.5 % Na_2SO_4 for complete precipitation, pseudoglobulin II. What then remains in solution is

regarded as albumin. It seems clear, that such a division, built upon the molar concentration of the electrolyte (the mentioned concentrations correspond to 1.0, 1.25 and 1.5 molar solutions) must give absolutely artificial limits between the »protein fractions». On the other hand the method seems to give reproducible results, when tested on large clinical materials representing different maladies.

I have used another and more specific of the old definitions for euglobulin i. e. the protein fraction, that is precipitated on dilution of the serum with water (or after dialysis against water). An old test for hyperglobulinemia has been based on this fact. A drop of blood is allowed to fall into water and it is stated if a gray haze develops. This test does not give any valuable information and I have tried another technique, that may give a chance of stating also qualitative differences in the euglobulin.

A simple method that may be used in any laboratory as an orientation for detecting pathological euglobulin is the following. One ml serum is mixed with 16 ml aq. dest. In the presence of euglobulin there forms a more or less massive precipitate, which is centrifuged down and washed with distilled water. The amount may be judged from the size of the precipitate, which is a rather approximate method, or by weighing. Some sera give a precipitate of the ordinary floccular type usually seen in proteins. Others show a practically limpid, highly viscous sediment that is obviously very hydrophilic. This sediment redissolves quantitatively in the presence of electrolytes. It is also possible to make a fractionated precipitation, as the euglobulins in some sera need higher electrolyte concentrations to keep in solution than others do. In Case I the euglobulin began to fall out already after the mixture of one ml serum with 6 ml water and the precipitation was complete in 1:10, with the formation of a very voluminous sediment and no further precipitation on dilution. In Case II there was beginning opacity also in 1:6 but the precipitation was not complete until 1:16. It is possible, that this means a real difference in the composition of the euglobulin and is not only a result of concentration. In some other cases of hyperglobulinemia there was found a slight precipitation in the dilution 1:2—3. Only in one case was there no decided precipitation before the dilution 1:9—10. The vast majority of cases with hyperglobulinemia did not show any precipitate

at all. Also in sera from a varying material of 80 cases with mixed diagnoses was there sometimes found traces of euglobulin but this was also present in the blood from quite healthy individuals and a slight opalescence with the formation of some precipitate is certainly not pathological.

The more chemical common methods for the determination of serum protein after *fractional precipitation* with Na_2SO_4 according to Howe and Kjeldalization of the precipitate are much to complicated to become really popular as routine methods in a hospital laboratory. For theoretical questions however they will probably be indispensable also in future. From many points of view the electrophoretical method, as it has been worked out by Tiselius, and the ultracentrifugation according to Svedberg are those methods that make the least damage to the protein molecule. In the three cases published here it has been possible to make such investigations on the sera. The results that seem to be of great interest will be published later on in greater detail by Doctor K. O. Pedersen.

Beside the methods, that have already been mentioned for the qualitative and quantitative examination of proteins there are a few phenomena that may lead to a diagnosis if they are correctly interpreted. The curious fact has been known for some time, that it may be impossible to count the erythrocytes as there appears a fine precipitate of globulins certainly caused by the mercury in Hayems solution. This has been wrongly mixed up with the auto-agglutination of the erythrocytes, that may sometimes be seen at temperatures below 37° (see Waldenström, 1942). This is quite another process caused by the action of an agglutinin at lower temperatures. It is not affected by the use of normal saline but only by warming.

Gros and Brockmann have used the flocculation of certain globulins in Hayems solution as a test for myeloma. I have tried the reaction in a number of cases with hyperglobulinemia but only once (in a patient with myeloma) found it positive. Its value therefore seems to be limited but a positive reaction probably indicates the increase of some special globulin fraction and may perhaps be used in future, when more is known about the chemical side of the question.

In the literature (cf for instance the excellent work by von Bonsdorff 1937) there are discussed a few other abnormalities in sera

from patients with myeloma. The deficient retraction of the coagulum may be very striking but as it is very difficult to interpret also in other conditions it may be hard to judge its real importance for the diagnosis. Another curious anomaly, that was first described by Wintrobe and Buell in 1933 and later by von Bonsdorff in 1937 and by Bing in 1940 is the division of the serum into two different strata, after standing in the ice chest. The lower is highly viscous and contains much euglobulin which may even be crystallized. The upper is more liquid and has not such a high globulin content. Also the coagulation of serum at 56° (on inactivation of the complement) has been discussed e. g. by Cantarow. Auto-inhibition for the Wassermann reaction (this anticomplementary effect has been studied in Sweden especially by Holmberg and Grönvall and by Olhagen) is another such phenomena.

Case II shows a clinical symptom that ought not at first to be regarded as a consequence of the hyperglobulinemia namely a *thrombosis v. centralis retinae*. The patient published by Wintrobe and Buell as well as one of Bing and Plums cases suffered from the same or a similar disease of the eyes and W. and B. regarded it as caused by the enormous agglomeration of the erythrocytes intravascularly. It is not easy to tell if the interpretation given by the American authors is correct but it does not seem impossible. Fåhræus has treated this question of capillary emboli from agglomerated erythrocytes already in 1921. The tendency to bleedings may also be a cause of intraocular changes (Bing and Plums case) as in case I, where extensive perivascular bleedings were found.

Beside the enormous increase in sedimentation rate the *anemia* is at first sight the most striking clinical symptom. In case III it was slightly hypochromic and the value for serum iron was somewhat lowered. The anemia was decidedly not caused by iron deficiency as a consequent iron therapy had no effect on the blood values. It is to be regretted, that the determination of serum iron is very uncertain in these cases as the protein precipitate is so massive, that it is difficult to obtain enough filtrate for a determination. The filtrate is also slightly opalescent. These complications may be suspected to work both increasing and decreasing the serum iron value and it is therefore best to state, that all determinations of the serum iron are uncertain in presence of considerable hyperglobulinemia.

The anemia is most decidedly not pernicious: hydrochloric acid in normal amounts was present in the gastric juice and the bone marrow did not show any megaloblasts in spite of severe anemia. In cases of myeloma a normochromic or slightly hyperchromic anemia is regarded as typical. Its explanation in this malady has simply been the crowding out of the normal marrow by the foreign myeloma cells. But also other explanations are possible. An essay to analyze the factors that might cooperate at the regulation of the normal erythrocyte level is not simple. A priori it seems probable, that the viscosity should play a part. When this is very much increased by the high globulin content it is possible that a decreased erythrocyte production may be an attempt at compensation. This is a mere conjecture but its validity should perhaps be tested in a suitable case.

The two patients case I and II have both shown a tendency to sickening bleedings from the gums and the nasal mucosa. These disturbances were even the reason, why the patients have come to hospital on their last visits. A determination of the coagulation time has shown, that it is decidedly increased. The quantitative determination of fibrinogen shows a considerable decrease and this ought to explain the retarded coagulation at least to a great part. This low fibrinogen content of the blood is probably the explanation of an observation that dr. Melander has made on the blood of this patient namely that a coagulum may dissolve spontaneously. I have myself once seen this in a patient with myeloma, who also showed protein precipitation when the blood was diluted with Hayems solution. The content of fibrinogen was not determined in this case. It does not seem probable, that the rather slight decrease of the platelet count should be of any influence on the coagulation if it is not assumed, that their quality is also changed. If it is the low content of fibrinogen or a low «quality» of the fibrinogen present or a deficiency in some other protein component necessary for hemostasis, that causes the haemorrhages in these patients the future will show. As it is it seems evident that large transfusions had a very favourable effect on the bleedings.

A third very striking change in the blood from these patients, when one has once observed it, is the quality of blood smears made on slides (see fig. 4). The first time when one of the nurses in the laboratory complained of the bad quality of the smears from case II we

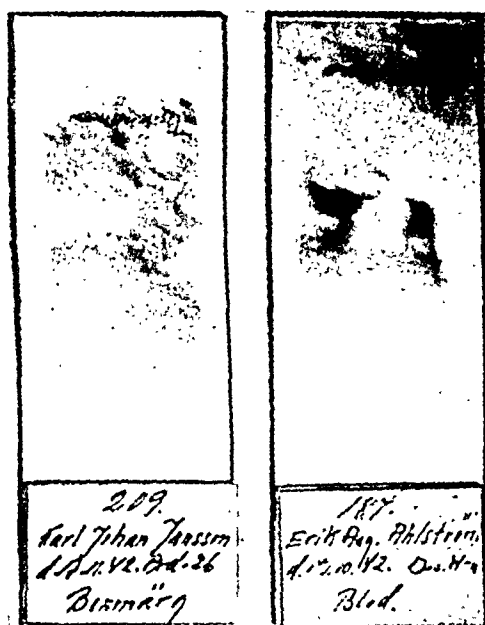


Fig. 4. Two slides with smears from blood (case II) and bone marrow (case I). Stained with May-Grünwald-Giemsa. Also slides from case III show similar changes but less marked, otherwise no such preparations were found among a large collection of sternal puncture specimens.

thought that it might possibly be a mere chance and depend upon a deficient cleaning of the slides. Several controls however showed that there was no technical fault. When it was later found, that also slides from Case I showed exactly the same picture, it was obvious that this change might have some diagnostic importance. Also in Case III older slides showed the same type. The change must be caused by the lowered stability of the protein film, that is spread and then dried and fixed on the slide. It is impossible to tell the cause of this as it has not as yet been possible to reproduce these changes experimentally.

This phenomenon has nothing to do however with a condition, that has been thoroughly studied in this Clinic, namely the autoagglutination of red blood corpuscles on cooling. Also in such cases the blood smears are of poor quality but this is caused by a clumping together of the erythrocytes through autoagglutination at temperatures below 37°C . This clumping and consequent streakiness of the slides is seen at once, not as is the case with the fragmentation of the blood smears from case I and II only after dyeing, fixation and staining.

Pathological protein fractions in the blood.

In 1848 Bence-Jones published the discovery of a new urinary protein. Since that time the study of definitely pathological proteins in different maladies seems to have made rather insignificant progress. The chief interest has been devoted to quantitative determinations of protein fractions and the work of Tiselius, Blix, Shedlovsky, Gutman and others on the electrophoresis of normal and pathological sera has much increased our knowledge in these matters. Of late the interest in the protein pattern of the serum has been very intense also in Scandinavia. This is probably partly explained by the important work on the physical chemistry of proteins, that has been done in the Institute of physical Chemistry in Upsala. (Prof. The Svedberg and Prof. Arne Tiselius).

In 1937 von Bonsdorff, Groth and Packalén published a very interesting case of myeloma in a man 37 years old. The patient had a marked hyperglobulinemia (7.5 %). When the serum was left standing at room temperature nothing happened but in the refrigerator there was seen a stratification with a highly viscous lower layer and an upper stratum with lower viscosity. The content of globulin was 10.7, viz. 5.3 %. In the lower stratum, where the euglobulin but not the pseudoglobulin had increased very much there was after some time found crystals. This crystallizing protein was examined very carefully from many points of view. It was precipitated as a globulin. The molecular weight was determined by K. O. Pedersen in the Institute for Physical Chemistry in Upsala and was found to be 200000 at ultracentrifugation i. e. somewhat higher than is found in the usual serum globulin fractions. Many facts favour the assumption, that this is not a normal component of the serum globulins. Nor is it a Bence Jones' protein, as the molecular weight of the latter is low (35—40000).

Packalén has later (1940) published one more case of myeloma with spontaneously crystallizing globulin.

Bing (1940) has described a case of myeloma with the same curious division of the serum on standing in the ice chest and he also believed that there were formed crystals in the lower layer.

Recently Holmberg and Grönvall have described a protein, that crystallizes readily from the serum under special conditions. The patient showed no signs of myeloma but she suffers from a

chronic arthritis. This protein is also a globulin with normal molecular weight (ultracentrifugation by K. O. Pedersen) and an electrophoretic migration that corresponds to the behaviour of a serum globulin. The γ -fraction was considerably increased but the pathological globulin (1.3 %) did not fit in with any of the previously described electrophoretic fractions. Also after electrophoretic purification did it give a positive Wassermann reaction. It is perhaps somehow related to the anticomplementary substance described by Olhagen, which seems to be increased not only in some instances of myeloma but also in chronic arthritis.

The three cases that have been published in this paper all show pathological proteins in the serum. Doctor K. O. Pedersen, who is at present working on the purification of these substances from the serum of Cases I—III has been kind enough to give a summary of his work. In the serum from case I there is found a fraction that seems homogenous on ultracentrifugation and gives a sed. constant at 20° of 19.2×10^{-13} corresponding to a molecular weight of over 1,000,000. In case II the sedimentation constant was of the same magnitude 20.6×10^{-13} . It is not easy to say, if this very high molecular weight is caused by an aggregation of globulin molecules or if there is really present a giant molecule. The decidedly monodisperse appearance on centrifugation rather speaks in favour of a preformed giant molecule.

Biologically of prime importance is the obscure question: What is the cause of these changes in the structure of the serum proteins? It must be stressed from the beginning that there is not only found a hyperglobulinemia but also at the same time a very marked lowering of the albumin to values at or below 2 %. In a large material of protein analysis in 102 cases with disturbances of the protein metabolism Gormsen only found 4 patients with a serum albumin value ≤ 2 %. None of these were instans of myeloma. The A : G quotient in my 3 cases thus changed not only through the absolute increase in globulin. Lowered albumin values are not uncommon in myeloma but I have never been able to find any explanation of this decrease. In case III there were certainly losses of albumin through the kidney during the last months as he had a picture resembling nephrosis but from the beginning he only showed slight albuminuria and this was absent or very slight in case I and II. Any really serious disturbance of liver function, that is otherwise

regarded as an important cause of hypalbuminemia, would be hard to reconcile with the clinical picture in general even if the high values for citric acid in Case II (but not in Cases I and III) might point to that organ. One explanation would be the assumption that there is some genetical connection between the albumin and globulin fractions of the serum.

I have not been able to find any description in the literature of a fibrinogenopenia associated with hyperglobulinemia as was found in Cases I and II. Probably this was the most important cause of the severe bleedings. The analyses of fibrinogen for instance in cases of myeloma, a malady, where the other globulin fractions have been investigated very closely in later years seem to be very few. Some authors have found an increase (v. Bonsdorff, Magnus Levy). If a decrease of fibrinogen may speak against the diagnosis myeloma, is not easy to tell at present judging from the very sparse material, that has been collected.

It might perhaps be expected, that this very rare but interesting and important symptom, a lowering of the fibrinogen content of the blood (inopenia) should be described also in other related or perhaps identical conditions. As a matter of fact such a finding is regarded as extremely rare in adults. Very low values for fibrinogen have in rare instances been noted as a congenital, hereditary error, experimentally after severe damage to the liver through chloroform or phosphorus and thirdly (by Jürgens and Trautwein) in a case where an extensive growth of cancer cells in the bone marrow was regarded as the cause of the inopenia. Perhaps somewhat rashly this last observation has been taken as a sign, that the bone marrow plays a part in the formation of fibrinogen.

Most characteristic for the first two cases published here is the very large amount of pathological globulin with the solubility of a euglobulin. The electrophoretic protarties in case II corresponded with a β -globulin (in case I electrophoresis was impossible because of the firm jelly, that formed at low temperature) but were not identical with this normal fraction. In case III there was also found a very high globulin content. On electrophoresis the fraction mostly resembled a β -globulin. In this case there was also present a component with very high molecular weight.

Discussion.

What may be regarded as the explanation of all these curious data? Why are these persons carriers of hitherto not described globulin fractions with giant molecules? If these are compared with other known proteins with a very high molecular weight we find, that their size most closely resembles some of the human antibodies e. g. against pneumonia (the specific pneumococcal antibodies in rabbits are considerably smaller, of the same size as ordinary globulin). It should be remembered, that a marked hyperglobulinemia is found (as an immune reaction?) in some probably infectious diseases of unknown origin e. g. in certain cases of lymphogranuloma benignum and in the virus disease lymphogranuloma venereum besides myelomatosis, that might be regarded as a more or less primary disturbance in protein metabolism. In lymphogranuloma venereum the protein fractions have been thoroughly studied by Gutman, Wise et al. Also in another chronic infectious disease namely Kala-azar a considerable increase in globulin has been observed.

May it be allowed to put forward another hypothesis as a possible explanation. We know from the experiments with plant viruses of different kinds, that the inoculation of an organism with a special virus may change a great part of the protein in the host plant into virus protein. This is e. g. a well established fact as regards tobacco mosaic virus. If a patient is «infected» with some virus protein it may be possible, that his own serum protein is transformed to virus protein instead. The hypothesis may possibly be tested experimentally on lymphogranuloma venereum, where it does not seem wholly improbable, that the pathological serum globulin is virus itself, which then ought to be contagious. It is also possible, that several conditions with a marked hyperglobulinemia are really chronic virus infections. In cases I and II the formation of virus protein might interfere with the synthesis of both albumin and fibrinogen. This seems more probable than the assumption, that the formation of some hypothetical antibody against an unknown infection should interfere with the production of such an indispensable substance as fibrinogen.

The danger of such a hypothetical virus infection would there-

fore not consist in any toxic action but rather in the virus forming a pathological matrix or inductor. The protein synthesis of the body thus runs along a pathological pathway and necessary building material is taken from the normal synthesis of e. g. albumin and fibrinogen.

In order to get a decided answer to all these questions it would be best to treat the problem experimentally e. g. with the infection of apes or monkeys with blood from these patients. It is to be observed however that only positive findings have any real value as proof, when we know how difficult the inoculation even with known virus diseases may be.

Summary.

The possible nature of myelomatosis as a disease of protein metabolism with secondary deposits in the bone marrow is discussed (cf the lipoidoses with high blood cholesterol and deposits in the tissues).

The author then gives a thorough clinical description of two patients probably suffering from a hitherto unknown disease in the bone marrow. A case that may possibly be interpreted as an instance of premyeloma is described (Case III). He shows longstanding hyperglobulinemia and severe anemia. In the bone marrow sternal puncture only revealed some plasma cells clustered together and never any roentgenological signs of myeloma. The course was progressive as regards the anemia. There was found a pathological globulin, but no inopenia and no hemorrhagic diathesis. Even at the post mortem the case remained obscure. There were not found any typical signs of myeloma.

Cases I and II showed signs of severe derangement of protein metabolism with very high globulin and low albumin values. There was also found a tendency to hemorrhages and low fibrinogen values. A severe normochromic anemia was noted. In both patients pathological euglobulins with very high viscosity, in one patient with a tendency to gelification of the serum at temperatures below +7°, were found. Obviously the molecule of this globulin must be a very large one. Studies in the ultracentrifuge and with the aid of electrophoresis by Doctor K. O. Pedersen in the Institute for

Physical Chemistry showed the presence of a globulin fraction with a very large molecule (mol. weight about 1,000,000) and a migration that did not correspond to any of the known globulin fractions. It lay next to the β -globulin.

The possible explanation of different anomalies in these patients: gelification of serum below $+7^{\circ}$, character of blood smears (see fig. 4), the high viscosity, the bleedings, the low albumin and the fibrinogenopenia, are discussed and related to each other. The causes of increased globulin content of the blood are treated and the hypothesis is put forward, that a mechanism analogous to the predominant formation of virus protein in a plant infected with e. g. tobacco mosaic virus may be present in these cases. A large amount of the protein in the blood would then be formed after the image of some abnormal »virus» protein, thus explaining the low values for the different types of normal protein (albumin and fibrinogen) and the very high molecular weight, that is of the same magnitude as that for virus proteins.

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A Case of Hyperglobulinemia with pronounced Eye Changes and Acrocyanosis.

By

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With reference to Jan Waldenström's paper on »Incipient Myelomatosis or Essential Hyperproteinemia» in this number of *Acta Medica*,¹ a brief account will here be given of a similar case which we have recently observed at the Central Hospital at Gävle. At the same time we desire to express our gratitude to Docent Waldenström for the valuable assistance rendered us when interpreting the case. For literature and discussion we refer to his paper mentioned above.

The patient is a factory-man, 50 years of age. A brother of his died of pulmonary tuberculosis 6 years ago. He himself was formerly a locomotive fireman and considered his sight to be better than that of most people. Since 1935 he has been troubled by fairly constant symptoms from various joints, especially the finger-joints, which now and again became swollen, stiff and sore, and were painful when moved. A doctor gave him medicine containing sodium salicylate, but the troubles were resistant to salicylic acid. As he did not consider that he could stand the medicine and as, furthermore, a patchy cyanotic discolouration of the skin on hands and feet began to appear in the autumn of 1935, which he ascribed to the medicine, he stopped taking it. Gradually there appeared a general livid cyanotic discolouration of the skin on his hands and feet, and this cyanosis has remained stationary since then. At the same time his hands and feet began to feel cold.

Tho these mild and fairly constant symptoms, which did not impair his capacity for work to any great extent, there was in April 1942 added a symptom that was more alarming and dramatic, for the patient was

¹ Vol. CXVII, fasc. III—IV.

then taken seriously ill with a temperature of 39°C and experienced pains when moving the shoulder-joints without any simultaneous swelling of the joints. The fever dropped in a week gradually to a subfebrile temperature and at the same time the articular symptoms disappeared. After another week the abdomen began to swell, however, and after a couple of weeks his sight was rapidly impaired, first on the right eye and after a few days also on the left. He was then unable to read, he could not even distinguish the features of those around him.

The doctors had diagnosed his disease as polyarthritis, but when the impairment of vision set in that diagnosis had to be abandoned, and he was admitted into the medical department of the hospital on May 30, 1942. The physical examination disclosed a seemingly healthy man of normal constitution. He had no oedema but a pronounced blue-violet discolouration of hands and feet. This acrocyanosis appeared, though less pronounced, also on other prominent parts of the body, e. g. nose and ears. The vision was good enough for finger-counting, but he could not distinguish the features of the patient in the neighbouring bed. The percussion note over the lungs was dull and the respiratory sound of the right base suspended. The heart presented normal physical findings. The blood pressure was 150/100 mm Hg. The abdomen was distended as in the case of a not too far advanced ascites, with distinct ascites configuration, a dull percussion note over the flank and waves of fluctuation. The liver was not palpable. The palpation findings *per rectum* were normal. Nothing objective from the joints. The neurological examination gave no positive findings. 4 days after admission the eyes were examined more thoroughly; the findings will be reported below.

Laboratory findings: *Urine:* Spec. grav. 1028, trace of albumen the first few days, then free of albumen the whole time except when last examined (Aug. 28, 1943), when there was again trace of albumen. Urobilin trace, Urobilinogen 0. Hammarsten's reaction neg., Diazo reaction neg. *Blood:* Sedimentation rate (SR) 62 mm/hr., percentage of hemoglobin (Hb) 90 %, red blood-corpuscles (RBC) 4.36 millions, white blood-cells (WBC) 11,800. In a stained blood film there were: Neutrophilic leucocytes 48 %, 16 % stabbs; lymphocytes 45 %; monocytes 4 %; eosinophilic leucocytes 2 %, and basophilic leucocytes 1 %. Wassermann's reaction was neg. in blood. Meulengracht value 4. *Faeces:* Nothing pathological. *Gastric juice:* Histamine-refractory achlorhydria. X-ray of cranium, lungs (after thoracentesis), stomach, small intestine, and colon gave negative findings. Tuberculin test: Mantoux pos. 10×10 mm with 0.002 g tuberculin. Blood culture: No growth.

During the first three weeks after admission the patient's temperature was subfebrile, about 38°C , but dropped gradually. He was then afebrile. The cyanosis varied greatly from day to day. Thoracentesis was performed on June 2nd, when 2,600 ml of liquid was drained. It was clear yellow and contained 4 % albumin with rare nuclear cells, monocytes. The guinea-pig test for tuberculosis was negative. The pleural exudate displayed no tendency to re-form.

In the beginning the patient's ascites appeared to be constant, and we were just considering the possibility of carrying out laparocentesis when to our surprise a spontaneous diuresis started which in the course of a week removed all ascites. His quantities of urine were then about 2,000 to 2,810 ml per day, and in 5 days a total of 12 l of urine was measured. The following entry was made on June 16th: »No ascites can be demonstrated any longer.»

As the first eye examination did not exclude the possibility of there existing thrombotic processes in the retinal vessels, 0.3 g of heparin was given daily during the first 9 days. The patient thought that his eyesight improved slightly as a result of the heparin injections, but the improvement could not be verified objectively.

While the patient was in hospital the SR presented increasing values: on June 24th it was 147 mm/hr and has remained high since then. The patient left hospital at midsummer, afebrile and free from symptoms except with reference to the eyes.

Since then we have now seen him every two or three months for more than a year. During this period he has felt quite well though his vision has been impaired, and in spite of temporary subjective and objective improvement it is on the whole unchanged. Control on July 23rd disclosed anemia (Hb 54 %, R.B.C. 2.3 millions, W.B.C. 13,600). SR 111 mm. Blood-sugar 70 mg %. He was given Tabl. Ferrofer forte, 6 daily, and on the occasion of the next control on Sept. 3rd Hb was 64 %, R.B.C. 2.84 millions, W.B.C. 6,400.

The SR being inexplicably high in spite of the patient feeling quite well, apart from his impairment of vision, a more comprehensive blood analysis was carried out on Nov. 5th, when the following values were obtained. Blood calcium 10.9 mg %, cholesterol 148 mg %, fat — 0.61 g of substance soluble in ether per 100 ml of serum, albumin 3.89 %, globulin 5.55 %, A/G 0.7, total protein 8.44. The analysis thus disclosed a remarkable degree of hyperglobulinemia and a slight hypo-albuminemia. For the sake of comparison we are quoting the normal values according to K. Liedholm, Svensk Läkartidning No. 23: 1943, for fibrinogen 0.2—0.5 %, for albumin 4.5—5.5 %, for globulin 1.5—3 %, the quotient A/G 1.5—2.5, and total protein 6—8 %.

In December 1942 the patient was again received into hospital for further examinations. The following data were then obtained. SR 46 mm, Hb 76 %, R. B. C. 3.88 millions, W. B. C. 6,600. In a stained blood film there were: R. B. C. normal; neutrophils 58 %, 20 % »stabs»; lymphocytes 37 %; eosinophils 1 % and monocytes 4 %. Thrombocytes 168,000. Blood-sugar 65 mg %, nonprotein nitrogen 34 mg %. No albumen in the urine. Kidney function test with 1 litre of water on an empty stomach: 850 ml secreted in 4 hours. Good dilution and concentration, spec. grav. 1,009—1,025. Liver function test with 40 g of galactose: Neg. result of reaction, i. e. 0.9 g was secreted.

Skeletal roentgen gave no indications of myeloma. Renewed blood analysis on Dec. 12th yielded the values: Albumin 3.37 %, globulin 4.61 %, A/G 0.7.

Table of certain clinical data.
For other values see text.

Date	SR mm/hr	Sahli %	R. B. C. mill.	W. B. C. 1000	Thrombo- cytes 1000	Blood protein				Alb. in urine
						Album. %	Globul. %	Total prot. %	A/G.	
1942										Trace
May 30	62	90	4.36	11.8						Neg.
June 5	70									"
June 24	147									"
July 23	111	54	2.30	13.6						"
Sept. 3	122	64	2.84	7.8						"
Oct. 29	23 ¹	80	3.74	6.4		3.89	5.55	9.44	0.7	"
Dec. 10	46	76	3.88	6.6	168	3.37	4.61	7.98	0.7	"
1943										
Feb. 17	90	73	3.76	7.8		2.68	4.50	7.68	0.6	"
May 10	92	74	3.98	6.6		3.80	3.97	7.77	0.9	"
June 26	85	67	2.44	8.2						Trace
Aug. 28	82 ²					4.00	3.55	7.87	1.1	"

¹ A technical mishap?

² On this occasion an electrophoretic analysis was made of the protein in the blood plasma, which yielded the following result: albumin 43.0 %, α -globulin 5.7 %, β -globulin 17.0 %, γ -globulin 29.9 % and fibrinogen 4.4 %. The analysis discloses a decided increase in the γ -globulin value as compared with that value in normal plasma, which is about 20 %.

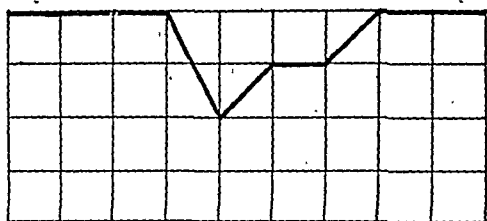
A/B 0.7, total protein 7.98 %, thus a considerably reduced albumin value in relation to an increased globulin value with a normal total protein value.

A control examination in Feb. 1943 gave the following values: SR 90 mm. Hb 73 %, R. B. C. 3.76 millions. In a stained blood film there were: R. B. C. normal; neutrophils 50 %, 50 % of which were «stabs»; lymphocytes 41 %, the majority being young or immature; monocytes 8 %; eosinophils 1 %. Blood analysis: Albumin 2.68 %; globulin 4.50 %; A/G 0.6; fibrinogen 0.68 %; total protein 7.68 %. X-ray of cranium, thorax and lumbar spine: No signs of myeloma.

On May 10th, 1943, a thorough examination was again made. The patient's general condition was good and he was subjectively well, apart from the eye symptoms. The cyanosis was unchanged as before. Since Dec. 9th, 1942, the patient is receiving Tabl. Priscol, 3 daily. He is very satisfied with this medicine, which he thinks has made his hands and feet feel warmer. SR 92 mm; Hb 74 %, R. B. C. 3.98 millions; W. B. C. 6,600. Sternal puncture was carried out and both bone marrow and blood smears were studied by Dr. N. G. Nordenson, who gave the following report.

Blood: Metamyelocytes 5.5 %; neutrophils 71 %, 20.5 % of which were «stabs»; lymphocytes 18.5 % and monocytes 5 %. Slight toxic changes. The red blood picture was on the whole normal. Bone marrow: Preparation rich in cells. The myelopoiesis slightly displaced towards the left and slight toxic alterations. Erythropoiesis on the whole normal. Megakaryocytes within normal range. Reticulum cells decidedly hyperplastic but comprise only the lymphoid forms, the plasma-cellular types having increased but slightly. They are not of the myeloma-cell type. Diagnosis: reactive marrow changes.

Also on the occasion of the last examinations in June and Aug. 1943 the patient's condition was found to be subjectively unchanged. It should be emphasized, however, that since the month of May this year he has experienced a feeling of numbness in the right side of the face, correspon-



Takata's reaction.

ding to the innervation area of the second branch of the trigeminus nerve, and this feeling remains unchanged. The only objective changes were a remarkable change in the albumin content of the plasma and the occurrence of traces of albumen in the urin.

No medication apart from the Tabl. Priscol mentioned above. The patient still feels subjectively well and only complains of his poor eyesight.

Among the numerous symptoms displayed by this patient, the eye changes are very remarkable. They are the symptoms that at present predominate.

The first eye examination was carried out on June 3rd, 1942, four days after the patient being admitted into hospital and about 5 weeks after the first symptoms appearing. What was then found was very uncommon and difficult to interpret. The eye symptoms have developed very dramatically. On May 19th he began to notice that his vision on the right eye disappeared during the course of the day. 3 days later he lost his sight in the other eye. When examined his vision in the right eye was 4/60 and in the left 3/60, and he could obtain this maximum vision only with peripheral fixation. No improvement could be obtained with glasses. The pupils reacted very slowly to light. The motility of the eyes seemed to be normal. The conjunctiva was pale with a slight chemosis in both eyes. All this was in itself not very remarkable. So much the more remarkable were the findings in the eye-grounds, however, which when first observed were described in the following manner (Karpe): »The eye-

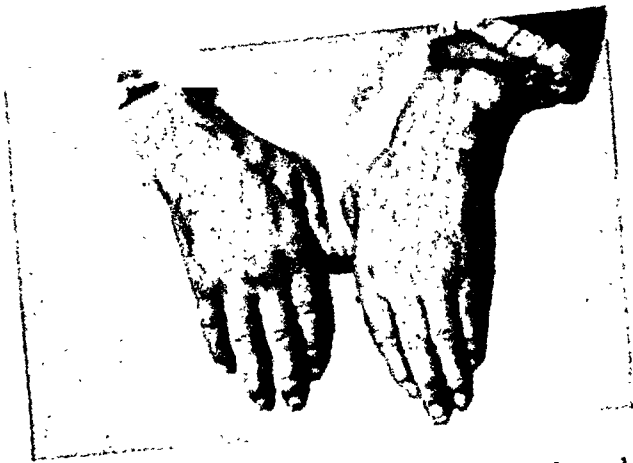


Fig. 1. The hands of the patient with the discoloured fingers.

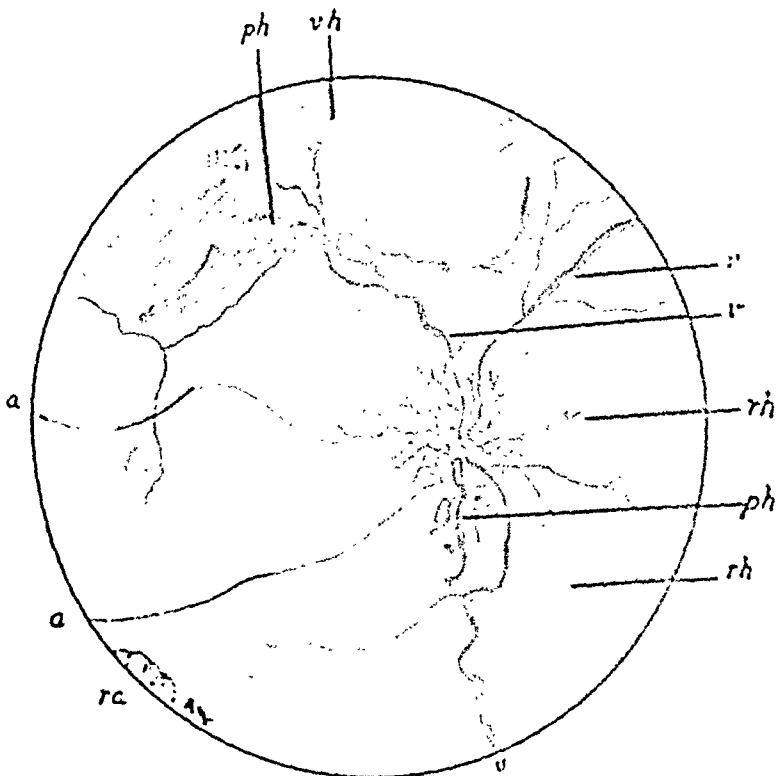


Fig. 2. The fundus of the left eye (reversed).
 a artery (?), v vein, rh retinal hemorrhage,
 ph preretinal hemorrhage,
 vh hemorrhage in the vitreous body,
 ra retinal arteriopathy,

grounds present a very peculiar picture with great retinal and vascular changes around the optic papillae and the maculae, the margins being normal. The vessels display great variations in size; the arteries are generally small, the veins dilated. Around the vessels and their branches the retina is whitish and swollen so as to form small centres grouped like ridges or wisps of clouds along the blood vessels. These symptoms are most strongly pronounced in the maculae, which are almost quite white, the foveae centrales being a contrasting cherry-red colour. Many of the small

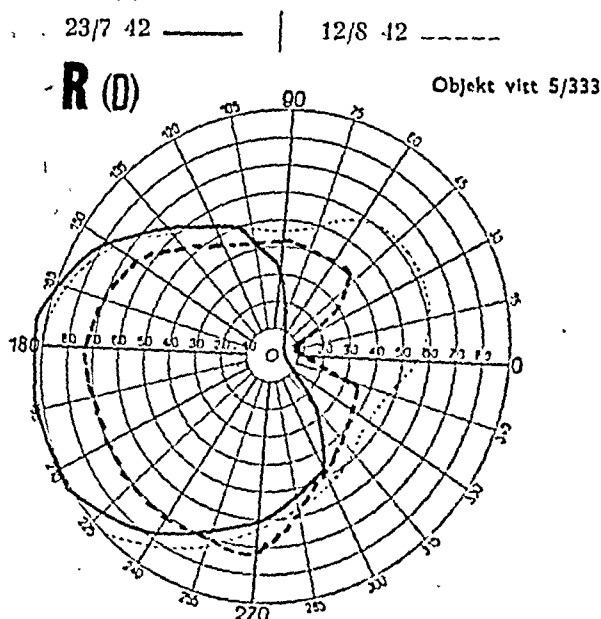


Fig. 3. Visual fields of the right eye.

veins in this area are distended and dark, and suddenly disappear as in the case of total thrombosis. The changes are somewhat more pronounced in the right than in the left eye. Solitary small haemorrhages also occur.

The findings are summarized as follows: "It seems to me that the picture indicates that the retina is the seat of multiple septic emboli and thrombi. Heparin treatment is suggested."

The patient's eye status was subsequently controlled on several occasions (Samuelson), the first time a week or so after the original examination, without any very great change being observed. What was especially striking, however, was the position of the changes close to the optic nerve and also the multiple small and light centres and haemorrhages, which latter were completely restricted to the retina and of the appearance, both as regards shape and size, found in the case of sepsis. The small white centres now reminded strongly of miliary tubercles in the retina.

When towards the end of July 1942 the eyes were again examined,

it was found that the patient's vision had improved somewhat both subjectively and objectively and that exudates and hemorrhages were being distinctly resorbed. Now, however, a fresh and rather uncommon phenomenon was noted, for the blood flow in the distended parts of the veins proceeded slowly and intermittently, like a regular flow of small beads between which there were yellowish white parts of the same size as the «beads». A couple of papilla-breadths from the papilla the vein again

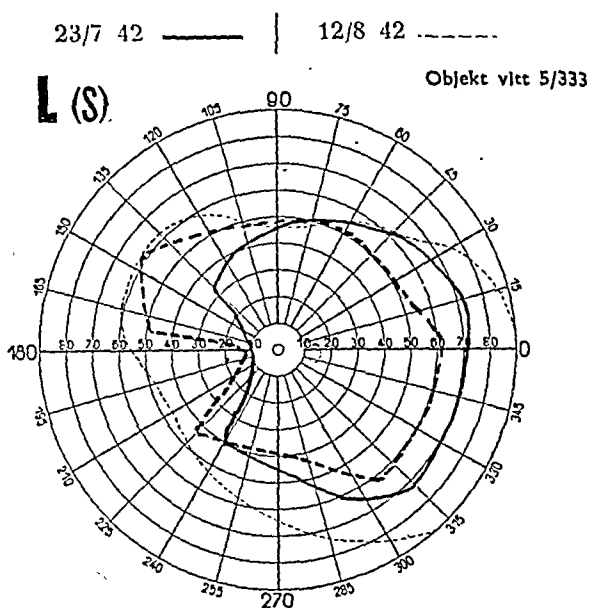


Fig. 4. Visual fields of the left eye.

narrowed rapidly and in this part, as in the more marginal parts, where the veins were also smaller, this phenomenon could not be observed. Such a symptom is rare and occurs in cases when the blood flow is retarded in vessels of different sizes in the same vessel. It arises only in the distended parts and can best be characterized as a dripping in the vessels instead of an even flow of blood. This does not imply that this is the only or even the right explanation. One possibility is that the changes in the plasma protein and the consequent increase in the agglutination of the blood corpuscles, which in its turn produces an increase in the rate of sedimentation (mirrored in the high SR), may in some manner have caused the phenomenon.

On this occasion an examination of the field of vision was also made, and a binasal incomplete hemianopsia was found to exist. This has subsequently increased in the left eye to an irregular reduction of the field of vision of no specific character, but in the right eye it remains practically unchanged (see fig. 3). No explanation of this change of the field of vision has been obtained, but it may perhaps be interpreted as caused partly by an initial withering away of the optic nerve, partly by the proliferous

changes in the retina and the vitreous body that appeared later and will be discussed below.

For some time no change was then observed, but that the vision increased to 4/50 in both eyes, still with peripheral fixation. In Dec. 1942 it was found, however, that hemorrhages had developed in both the eye-grounds. The patient had observed, too, that his eyesight had become poorer during

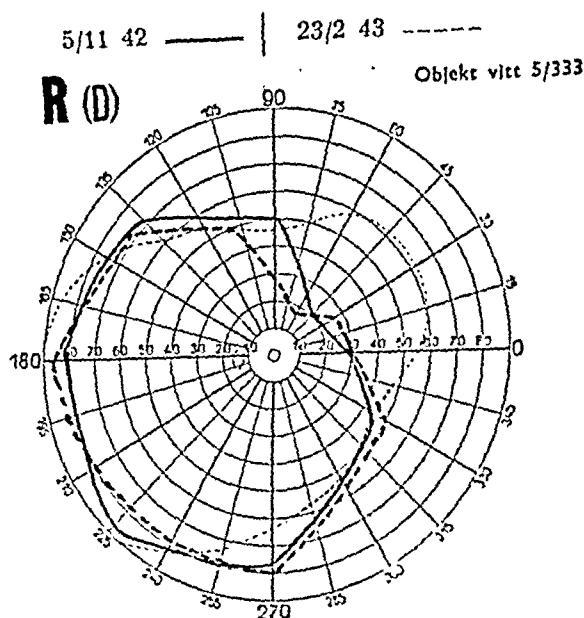


Fig. 5. Visual fields of the right eye.

the last few days. The hemorrhages appeared to have started in the veins running downwards, whose irregular width had remained constant the whole time. In the right eye there were only retinal and preretinal hemorrhages, but in the left eye a very great part of the preretinal hemorrhage had also invaded the vitreous body. As the hemorrhages were fairly marginal they did not obstruct the vision very much, but produced a certain subjective feeling of obscuration in the field of vision. Instead the eyesight increased somewhat in the right eye, to 0.1, where, too, the hemorrhages were not so pronounced, but in the left eye it was 3—4/50. No improvement could be achieved with glasses.

In the control examination on Feb. 1943 it proved that the exudative centres as well as the original retinal hemorrhages in the fundus of the eye had been partly resorbed, and now there was seen a light grey-white-rosy atrophy in the retina, comprising the whole macular area and an area of about the same size outside it. The optic papillae were very pale with diffuse margins and an intense new formation of fine vessels within the papillae. These vessels seemed to emanate from the veins, for the arteries were so greatly constricted that they could not be distinguished in the altered parts of the papillar area. In certain parts peripherally

to the macular area they seemed to have been replaced by »silver arteries». The above described peculiar changes in the blood flow in the distended parts of the veins could no longer be observed. This would seem to depend on the fact that the blood flow was smoother and is interpreted as an increase in the amount flowing through, possibly as a result of the treatment (Priscot). The haemorrhages in and on the retina and the hemorrhages in the vitreous body were on the whole unchanged. The whole

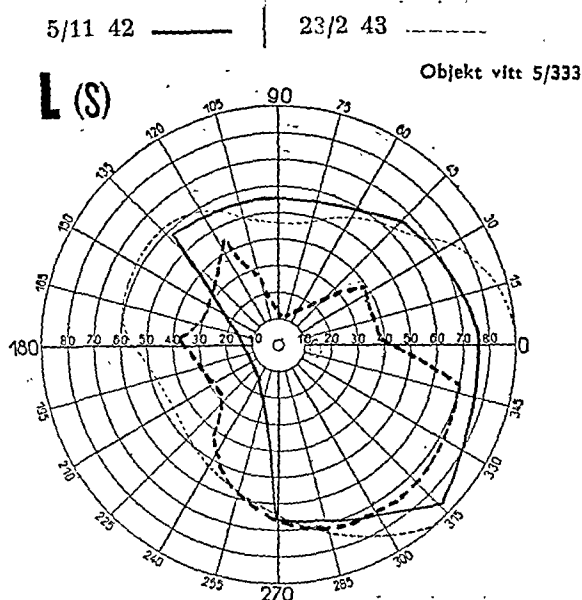


Fig. 6. Visual fields of the left eye.

vitreous body had, however, been made somewhat turbid, so that the fundus of the eye was slightly dimmed, though not more than that the details could be made out (the sketch was made on this occasion see fig. 2).

The patient's condition was now thought to be fairly definitive, especially as during the last 4 months he had felt comparatively well and had only complained of his poor eyesight. However, he was still examined from time to time and in the beginning of May 1943 we were surprised to find that his eye status had deteriorated due to some very curious changes in the eyegrounds and vitreous bodies. This was especially true of the left eye, where the vision was now but half of that of the other eye, which was 4—5/50. This vision was still obtained with peripheral fixation only, but now slowly than before.

The changes consisted partly of dense veil-like new formations mainly on the site of the former hemorrhages and were interpreted as a form of *retinitis proliferans*, partly of fan-shaped and omentum-like formations all emanating from the optic papilla or at any rate from its immediate vicinity. Also these latter reminded of *retinitis proliferans*. The remarkable thing about them was, however, that they contained new blood vessels,

partly at the edge, partly (and that especially in the last-mentioned types) also between the margins, which caused these latter even still more to resemble spread-out omentum. Here and there small hemorrhages could also be noted in and on the «veils».

In one eye one of the new formations had a cystic appearance, it being possible to see new blood vessels at different depths as in a transparent sphere. A strong impression was gained, however, that all this was only different expressions of one and the same intense proliferative process.

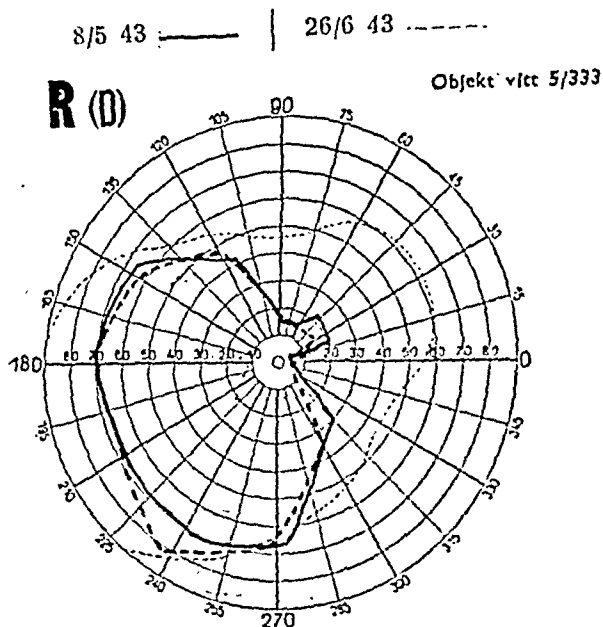


Fig. 7. Visual fields of the right eye.

In the left eye it was so pronounced, that the optic papilla was almost entirely obstructed.

Except for these symptoms, of which we had no acceptable explanation, we could only establish an increasing defect of the field of vision of the left eye. The eye status otherwise was essentially normal. Thus the position and motility of the eyes was normal, but the convergence ability was lacking. The pupils were certainly comparatively large, but alike on both sides and reacted to light directly and consensually, though it could not be determined whether this was for the sake of accommodation. These symptoms have, what more, remained unchanged during the whole period of observation. Likewise it has not been possible to establish any pathologic change in the eye pressure; neither a rise nor a drop.

About 6 weeks later (June 1943) some of the changes last described were being resorbed, this being especially true of the hemorrhages but also, though much less so, of the new formations resembling *retinitis proliferans*. On the other hand the blood vessels displayed a lively proliferation and from the earlier blood vessels at the edge of the new formations vessels

of various lengths were now seen to run as straight as nails into the vitreous body (?). The vision was now somewhat better in the right eye, but otherwise no change could be observed.

When after another few weeks (Aug. 1943) the eyes were again examined, a considerable impairment was found to have set in, especially with reference to the left eye. The haemorrhages already described now appeared to have penetrated into the central parts of the vitreous body and had spread so very much that it was no longer possible to see any details

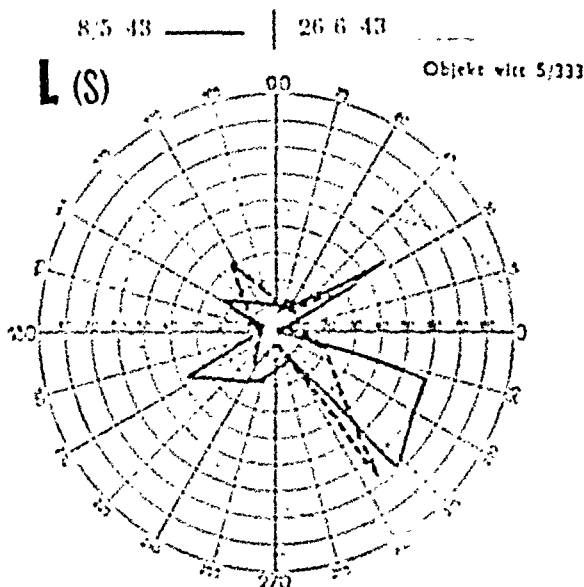


Fig. 8. Visual fields of the left eye.

of the eye-grounds. The patient's vision had also deteriorated, so that he was merely able to perceive light peripherally and locate light coming from below. The new vessels formed in the *sommentum* were now still more pronounced and were greatly ramified like a delta. The same kind of changes were observed in the right eye though they were less pronounced. The vision was somewhat better here, too, being 3—4/50. The eye pressure remains normal.

It is evident that the disease has not yet come to a standstill, either as regards the eye- or the general symptoms, and the case is still being observed.

Summary.

A middle-aged man, who during a number of years has been troubled by fairly mild and constant symptoms from the joints of a polyarthritic nature, has an acute attack of a severe sepsis-like infectious disease with a high temperature, high SR, increased joint

symptoms, rapid impairment of vision, moderate dyspnoea, and an enlargement of the abdomen. In addition to a striking and very pronounced acrocyanosis (which, however, he has suffered from for the past 8 years) the examination discloses a series of remarkable eye changes in the retina, retinal vessels, optic nerve and vitreous body, which are difficult to interpret. The arteries were extremely small, some of them being obliterated («silver arteries»). For some time the veins close to the papilla were varicosely distended, being narrower farther peripherally. The blood flow was fractured in a curious and uncommon manner, which phenomenon gradually ceased. In the retina there were to begin with grey cloudy exudative centres, which disappeared. There were also retinal hemorrhages and later preretinal hemorrhages appeared as well as hemorrhages in the vitreous body, which when resorbed were replaced by new formations of the type of *retinitis proliferans* but first of all by an uncommonly intense new formation of vessels, partly within the strings in the vitreous body and partly outside and apparently free inside the vitreous body. The optic nerves gradually became atrophic here too, with a profuse new formation of small vessels. The vision was strongly impaired and the patient was but able, with peripheral fixation, to count fingers at a distance of three metres with one eye and had only peripheral perception of light with the other; the field of vision was restricted, most in the left eye, probably mainly on account of the atrophy of the optic nerve.

Other findings were a large pleural exudation on one side, ascites, high SR, leukocytosis with an increased number of *non-segmented* leukocytes, and lymphocytosis. The gastric juice contained no hydrochloric acid. The patient soon became afebrile and the pleural exudation showed no tendency to re-form after draining. An enhanced spontaneous diuresis was accompanied by the rapid disappearance of the symptoms of ascites. Apart from the remaining impairment of vision the patient felt quite well. However, he still has a very high SR. An anaemia appears but vanishes upon the patient being given iron. Blood analysis discloses hyperglobulinemia combined with a decreased albumin value. The changes in the blood picture remain the same during the whole observation period, more than a year.

Wintrobe and Buell have described a case somewhat similar to

ours (Bulletin of the Johns Hopkins Hospital 52: 1933). That case also presented hyperproteinemia, acrocyanosis and changes of the eye-grounds. The latter, however, were evidently not attributed very great importance; in any case they are described very briefly: »The eye-grounds showed extensive hemorrhages with extreme dilatation of the veins, findings which were interpreted as indicating thrombosis of the central veins of the retinae. The arteries were slightly smaller than normal but definitely patent.« A spontaneous fracture disclosed bone metastases. Death occurred after 4 months' observation. The diagnosis of the pathologists was plasma-cell myeloma invading the humerus and lumbar vertebra.

In our case it will with great probability be possible to exclude myeloma. The decidedly chronic course (we have observed the patient for more than a year and the cyanosis has lasted for 8 years), the absence of Bence-Jones protein in the urine, the absence of myeloma cells in the tissue removed by sternal puncture, and repeated negative skeletal roentgen examinations decidedly contradict such a diagnosis. Probably we are confronted with an unknown syndrome where the clinical picture is dominated by the triad: hyperproteinemia, acrocyanosis, and changes in the fundus of the eye. We do not even know however, whether the hyperproteinemia is the cause of this circulatory disturbance or whether it is the other way round. Perhaps the two symptoms are collateral. The variations in the changes in the eye-grounds seem to show that it is a disease in progress. Perhaps the continued study of this case will contribute to the solution of this problem.

From the Psychiatric Department of the Clinic for Psychiatry and Neurology, Utrecht (Holland). Director: Prof. Dr. H. C. Rümke.

On spontaneous hypoglycemia referable to the presence of adenomas in the islets of Langerhans.

By

S. LUPS.

(Submitted for publication, January 5, 1944).

The diagnosis: spontaneous hypoglycemia, tentatively put forward to account for certain psychical and autonomic-nervous symptoms, requires for its confirmation the presence in the patient of a low blood-sugar value and a prompt response to the administration of sugar.

Hyperinsulinism is not the only cause of spontaneous hypoglycemia. An abnormally low blood-sugar value may also be due to other causes. In order to arrive at an effective therapy, the diagnosis: spontaneous hypoglycemia is therefore insufficient and must be supplemented by an inquiry into the nature of the hypoglycemia.

Conn¹ in studying the most important information in the literature on spontaneous hypoglycemia arrived at the conclusion that 80—90 per cent of the cases may be classified under the 3 following headings:

- I. Organic spontaneous hypoglycemia.
- II. Functional spontaneous hypoglycemia.
- III. Hepatogenic spontaneous hypoglycemia.

¹ Conn, Jrn. Am. Med. Ass. vol. 115—1940—p. 1669.

The remaining 10—20 per cent are associated with hypofunction of the anterior pituitary gland, of the thyroid and of the adrenal cortex and further with renal glycosuria, with strenuous muscular exertion and with pregnancy and lactation. The three initially mentioned groups being in general practice the more important ones, we will confine ourselves to them. For more complete information the reader is referred to Conn's paper.

Group I. Organic hyperinsulinism. In this category the following cases are especially noteworthy: pancreatic-islet-cell adenoma, pancreatic-islet-cell carcinoma and hypertrophy of the islets.

Conclusive evidence for the presence in the pancreas of one of these anatomic aberrations may be derived from:

a. The regular occurrence of fasting blood-sugar levels below 0.05 per cent. The attacks due to the hypoglycemic condition take place in the morning before breakfast, 3—4 hours after meals and also after strenuous bodily exertion.

Akerberg¹ is of opinion that the morning attacks are not merely due to fasting, but should also be ascribed to the circumstance that the liver at this time is in the assimilatory phase, in which the synthesis of glycogen takes place and in which the compensatory glycogenolysis is therefore slowed down.

b. A decrease of the fasting blood-sugar level below 0.04 per cent, when a diet low in carbohydrate is given.

(In Group III too on a normal diet fasting blood-sugar levels below 0.05 per cent and on a diet low in carbohydrate below 0.04 per cent may occur.)

c. An exceedingly low blood-sugar level during the attacks.

d. The absence outside the pancreas of factors which might cause hypoglycemia.

Carcinomas starting from the pancreatic-islet-cells (like the one described by Wilder et al.²), appear to be comparatively rare. Van Beek³ in studying the literature found but seven cases recorded. (Ordinary pancreas carcinoma, which according to Vos⁴ is found in one half per cent of all post mortem examinations, origi-

¹ Akerberg, Acta Chir. Scand. vol. 83—1940—p. 104.

² Wilder, Allen, Power, and Robertson, Jrn. Am. Med. Ass. vol. 89—1927—p. 348.

³ Van Beek, Ned. Tijdschr. v. Geneesk. 86—II—1942—p. 1045.

⁴ Vos, Ned. Tijdschr. v. Geneesk. 1940—II—p. 2220.

nates in the excretory part of the organ and causes no hypoglycemia.)

Pancreas tumors causing hypoglycemia are, as a rule, proliferations of the islet cells of an adenomatous nature.

Clinically the differentiation between the islet-cells adenoma and the islet-cells carcinoma is very difficult and often even impossible. The patient's age and the speed with which the symptoms proceed would not give any indication of the differentiation (Windfeld¹).

With regard to the importance in practical medicine to be assigned to the islet cells hypertrophy, opinions diverge. Whether hypertrophy of the islet cells occurs in adults, is uncertain and becomes more and more doubtful.

Group II. Functional hyperinsulinism. To this group the great majority of the hypoglycemias belong. Its most important symptoms are:

- a. The absence of the progressive tendency found in group I.
- b. That periods of serious complaints alternate with periods of complete recovery.
- c. The usually normal fasting blood-sugar level and accordingly the absence of attacks in the period before breakfast.
- d. That a diet low in carbohydrate causes a lowering of the fasting bloodsugar level, which is not in excess of the decrease found in normal persons.
- e. The occurrence of hypoglycemic attacks 3—4 hours after meals.
- f. The short duration of the attacks and the usually spontaneous recovery of the patient, even if no food is administered.

A diet high in carbohydrate usually increases the severity and the frequency of the attacks.

Clark and Green² noted a remarkable improvement when a diet low in carbohydrate but high in fat was given. According to John³ this kind of hypoglycemia reacts favourably on a diet high in fat, when 20 I. U. of insulin are given half an hour after each meal. Conn and Wilder⁴ saw good results of a diet high in protein and low in carbohydrate.

¹ Windfeld, *Acta Chir. Scand.* vol. 84—1941—p. 155.

² Clark and Green, *Proc. Soc. Exper. Biol. and Med.* vol. 32—1935—p. 1459.

³ John, *Endocrinology* vol. 19—1935—p. 689.

⁴ Conn, *Jrn. Clin. Investigation*, vol. 15—1936—p. 673.

Group III. Hepatogenic hypoglycemia. It is characterized by:

a. A decided tendency to progressive development.

b. The frequent occurrence of post-prandial hypoglycemia.

Especially during the night it is difficult to maintain a satisfactory blood-sugar level. As the decrease in the amount of blood sugar continually proceeds, the minimum value is reached in the early morning before breakfast; it is at this moment that most of the attacks occur. The frequency and severity of the attacks increase when the amount of carbohydrate in the diet is reduced.

c. The form of the dextrose tolerance curve, which notwithstanding the exceedingly low fasting blood-sugar level, resembles that found in cases of diabetes.

Whipple¹ is of opinion that in this category serious disturbances of the liver function are always noticeable. According to Conn, however, disturbances of the liver function or disease of the gallbladder are not necessarily revealed by clinical investigation. At any rate, since the disturbance of one of the liver functions need not impair the normal conduct of the numerous other ones, as many of them should be tested as appears practicable.

Adenomas of the islets of Langerhans have but rarely been recorded. In Dutch medical literature there is a communication by Vos, dealing with a post-mortem examination. Moreover a paper has been published by Van Beek, Haex and Kooreman² in which a. o. a case is described where an operation was performed. Another case, in which the patient was operated upon, follows here.

Van G., a man aged 28, was taken to the Clinic for Psychiatry on Nov. 6, 1941, with the following anamnesis:

Nine years ago, at nine o'clock in the morning of December, 31, he had for the first time a «nervous attack». Shortly before he had behaved in a strange way. In putting on his clothes, he had made mistakes, his hands had been shaking and when questioned, he had not answered. Afterwards he felt oppressed, in bed he moved to and fro and vomited slightly. At ten o'clock he fell asleep and slept without a break until four o'clock next morning. During this sleep he opened his eyes when spoken to, but gave no answer. The day after he felt somewhat dull, but the next day he resumed his work. Three weeks later, when bicycling, he had a similar attack. He fell down and just when it occurred to him that people were shouting, he lost consciousness. He soon came to again and mounted his bicycle once more. A month later, when at work, he suddenly felt

¹ Whipple, Jrn. Int. de Chir. T. 3—1938—p. 237.

² Van Beek, Haex en Kooreman, Geneesk. Bladen. 1942.

giddy, experienced an irritating sensation in both hands, which lasted for about ten minutes, but did not lose consciousness. During this period he was treated with luminal, but without success.

Afterwards about ten times a year slight attacks manifested themselves, which started with a hungry feeling, whilst his hands trembled and his arms made jerky movements. This was followed by a period of mental confusion of which he had no recollection afterwards. Sometimes, however, the attack restricted itself to the first-mentioned symptoms. It also occurred that he saw stars before his left eye and jerked his left arm. These symptoms were attended by a sensation of hunger. If the patient at the beginning of an attack found a opportunity to eat something, he often succeeded in checking its further development. After an attack too he felt hungry. Ten months ago and again two months later, the patient was sent to hospital and taken into observation by a neurologist. As a cerebral cause suggested itself, lumbar puncture and ventriculography were applied, but no deviations were found. During the two periods of observation, which between them took up eight weeks, no attacks were noticed. Since his last stay at the hospital the patient took 250 mg luminal and 2 g bromide daily, but notwithstanding this treatment the attacks did not cease.

This time the patient asked for admission, because in the six preceding days every morning, when about to rise, he had had a rather severe attack, which lasted respectively for 1, 2, 1½, 2, ¾ and 1 hours. After these attacks he took his bicycle and went, as he was used to do, to his work.

Patient was admitted at 4 o'clock in the afternoon. Since the early morning he had been in a somnolent condition. When spoken to, he awoke more or less and answered very slowly, but his speech was almost inarticulate and at times nearly unintelligible. When admonished to speak more clearly, his speech became somewhat more intelligible, but the end of his sentences were always lost in indistinguishable sounds. He was insufficiently aware of time and space. He was able, however, to recognise the people who were with him and also fairly sure of his own personality. His complexion was rubicund. In bed his head fell back limply and his mouth partially opened; he wanted to sleep and asked to be called early, because he must go to his work. Later he imagined himself to be felling trees with his comrades.

The physical examination revealed no abnormalities. Patient was slenderly built and had but little subcutaneous fat; the tension was 150/90.

The morning after his admission the patient was semi-comatose. The blood-sugar level was found to be 0.047 per cent and was therefore hypoglycemic.

The next day the following blood-sugar values were found: fasting 0.061, 8 a. m. 0.057, 9 a. m. 0.077, 10 a. m. 0.081, noon 0.061, 1. p. m. 0.097, 4 p. m. 0.076, 9 p. m. 0.115 per cent. On the following days similar values were obtained. The dextrose tolerance test with 50 g dextrose gave the following values: 0.048, 0.120, 0.048, 0.036 and 0.036 per cent. About twice daily, especially before breakfast, but sometimes also later in the morning or in the afternoon, patient became slightly somnolent and

tossed restlessly about, a condition which usually passed into delirium. During these attacks, which never occurred after 7 o'clock in the evening, the blood-sugar values varied between 0.061 and 0.044 per cent. As the attacks were always immediately checked by the administration of sugar, the diagnosis was: spontaneous hypo lycemia. During this time the fasting blood-sugar values were: 0.068, 0.059, 0.065, 0.066, 0.048, 0.061, 0.070 and 0.061 per cent. As patient, contrary to our advice, returned home, a more detailed diagnosis could not be made.

At home the attacks continued and already one week later patient was sent by the family doctor to surgeon Dr. Klinkenbergh, who took him to St. Anthony Hospital and asked me to complete the diagnosis.

During this period of observation too, hypoglycemic attacks of the same kind as those observed in the Psychiatric Clinic occurred.

Differential diagnosis: As no indications were present for a lesion of the hypophysis (Simmonds's disease, hypophysis tumor) or for a lesion of the central nervous system, the only possibilities between which the differential diagnosis had to decide, were: hyperinsulinism due to a lesion of the pancreas, functional hyperinsulinism, hypofunction of the adrenal cortex and a lesion of the liver.

Although the amount of hippuric acid in the urine (2.8 g instead of 4.4 g in 4 hours) must be regarded as insufficient, a hepatic disease seemed out of the question: the galactose tolerance was normal, urobilin was absent from the urine, there was no macrocytosis in the erythrocytes, the bilirubin content of the serum was not increased and above all the blood-sugar curve did not rise above 0.120 per cent and the gallbladder was normal and contained no stones.

A lesion of the adrenal cortex was not so easily dismissed, the difficulty being that the blood contained almost no adrenalin (less than 10^{-15} , whereas normally 10^{-13} is found, dr. Le Heux). However, as there were no indications for Addison's disease or for the presence of an adrenal tumor, this possibility too was finally rejected.

A choice had now to be made between the two remaining possibilities: the presence of a pancreas adenoma and functional hyperinsulinism (on account of the protracted period of the complaints, the presence of a pancreas carcinoma seemed unlikely). The fasting bloodsugar levels, which for the discrimination between these two kinds of hyperinsulinism are, as a rule, conclusive evidence, were of no avail here. On a normal diet they varied between 0.046 and 0.074 per cent, with an average value of 0.055 per cent (based on 18 determinations); on a diet containing 20 g carbohydrate, 50 g protein and 137 g fat, they sank to 0.047, 0.044, 0.040 and 0.046 per cent. the attacks becoming at the same time more severe. Although during this period, when a normal diet was given, the fasting blood-sugar values seldom sank below 0.050 per cent and a provocative diet gave no values below 0.040 per cent (cf. sub Group I), a pancreas exploration was finally decided on. We were chiefly led to this decision because throughout the day low blood-sugar values were found, a condi-

tion which we had never noticed in cases of functional hyperinsulinism and also because the disease showed a progressive tendency.

The operation (Dr. Klinkenbergh) revealed the presence of two adenomas. The operation report follows here:

Shortly before operation patient received 50 cm³ of a 20 per cent dextrose solution intravenously. The operation took place under aether narcosis (open mask) after a preliminary treatment with aethylchloride. A transverse, slightly arcuate incision was made in the upper part of the abdomen, a few cm above the navel. The two muscoli recti with their sheaths were transversely cut, the incision extending slightly into the lateral abdominal muscles. After the peritoneum had been opened, liver, gall-bladder, stomach, colon and spleen were inspected and palpated. No aberrations were found in these organs.

After the branches of the arteriae gastro-epiploicae dextra et sinistra had been tied up, the ligamentum gastro-colicum and the ligamentum gastro-splenicum were split over their whole lengths. In this way the bursa omentalis was opened and the whole pancreas revealed.

The pancreas appeared normal in colour, size and consistency, but in the cauda, which lay very deep, close to the spleen two small tumors were visible on the surface, one in the midst of the parenchyma and the other in the lower part. The former was the size of a cherry, the latter that of a small marble. Their colour was not markedly different from that of the rest of the pancreas (they were neither red nor purple), but they were easily recognizable by their globular shape and by their consistency. Some small bloodvessels were first of all tied up, and then the tumors were easily detached and removed. In the wound bed too a few small vessels had to be seized and tied up, but the bleeding was of no importance. The whole pancreas, especially the caput was once more carefully inspected and palpated, but nothing suspicious was found. In the wound bed a drain was laid, which through a stabwound in the wall of the abdomen opened on the surface. The pancreas capsule was too thin to be sewn up and the ligamentum gastro-colicum too was left as it was. The incision in the abdominal wall was carefully closed, the layers one by one being sewn up. As the cauda pancreatis lay uncommonly deep, apart from the transverse incision, part of the linea alba had to be cleft as well.

An extensive median laparotomy would have made this operation easier than the arched incision recommended by American authors, (a. o. Whipple), practised in this case.

After the operation patient received a transfusion of half a liter of citrate blood (dripping infusion).

About 10 minutes after the patient had been taken back to the ward, convulsions set in, which an intravenous injection of 40 cm³ of a 20 per cent dextrose solution and of adrenalin was unable to check. After the administration of a mixture of carbon dioxide and oxygen the condition improved.

From the drain about 25 cm³ of an at first bloody, afterwards more watery fluid was obtained. After two days the flow came to a stop and

the seventh day the sutures were removed, the wound healed without any complications. On the fourteenth day patient was allowed to leave his bed and nineteen days after the operation he returned home completely cured, and up to now (October '43) no other hypoglycemic attack has manifested itself.

Histological investigation.

The smaller tumor has been studied by Miss Ca van Beek in the laboratory of Prof. Lignac. Her report is here reproduced:

Macroscopical results:

The object is a piece of more or less solid tumor tissue, which shows through a firm, locally vascular fibrous capsule of a greyish hue. The cross-section is homogeneous of a greyish colour, here and there with yellowish spots and traversed by septa springing from the capsule.

Microscopical results:

The layer of pancreas tissue outside the thick fibrous capsule is markedly compressed. The excretory parenchyma is atrophic and the same is true of the islets of Langerhans, which are normal in other respects.

Strongly oedematous and therefore rather thick vascular septa of connective tissue extend from the capsule into the mass of tumor cells. Hyalin had nowhere been precipitated and the amyloid reactions too are negative.

In the frozen sections the tumor tissue reminds one of liver parenchyma, but in the celloidin sections this resemblance is less clear. It consists of numerous polygonous cells with large, sometimes globose, but usually ovoid, vesicular nuclei. When stained with haematoxylin the latter become purple and show a fine network of chromatin, between which one or two nucleoli are clearly perceptible. Not rarely two nuclei are found in one cell.

In several places the protoplasm is minutely vacuolised. This was suggested already by the macroscopical aspect, for the yellow spots are due to the presence of fat and/or lipid; when stained with Sudan III, numerous cells appear to be filled with small orange globules.

The tumor cells vary in size and shape. Necrosis is nowhere seen and mitoses are a great exception. There is no infiltration into the surrounding tissue and the blood vessels contain no tumor cells. Here and there the fibrous stroma is somewhat more strongly developed and encloses islets of tumor tissue; the peripheric cells of the latter show a more or less distinct palisade formation.

Between the tumor cells numerous, usually collapsed, capillaries are found.

A few slight extravasations are observed.

Conclusion: a benign adenoma of the islets of Langerhans.

A small piece of the larger tumor was examined by Prof. Nieuwenhuys. Its structure too answers that of the adenomas of the islets of Langerhans. There is a large amount of connective tissue and the epithelium is split up into very irregular islets. Here too no signs of malignancy were observed.

The details given above refer to a man, 28 years of age, who for nine years had been suffering from hypoglycemic attacks, but whose disease during the first eight years showed no marked progression. Only in the last months a deterioration set in and the frequency as well as the severity of the attacks increased.

In working out the differential diagnosis some difficulty was experienced when a choice had to be made between the presence of an adenoma and functional hyperinsulinism. On a normal diet the fasting blood-sugar values were on the average 0.055 per cent, and on a diet containing 20 g carbohydrate, 50 g protein and 137 g fat 0.046 per cent was found. As Conn concludes from the study of an extensive material that the fasting blood-sugar level in cases of an adenoma, usually lies with a normal diet below 0.050 per cent and with a diet low in carbohydrate but high in fat below 0.040 per cent, our data seemed to make the presence of an adenoma less probable than that of functional hyperinsulinism. *As throughout the day low blood-sugar levels were found we were, nevertheless, convinced that a pancreas adenoma must be present also on account of the progression which the disease of late had shown. Operation indeed revealed the presence of two benign adenomas starting from the islets of Langerhans; they were found in the cauda pancreatis.*

Some peculiarities observed in this case deserve a short discussion.

The basic metabolic rate was in this patient minus 13 per cent and the respiratory quotient 0.85 (Prof. Jongbloed). In the cases recorded in literature, the respiratory quotient is, on the whole, high, which means that a large amount of sugar is oxidized.

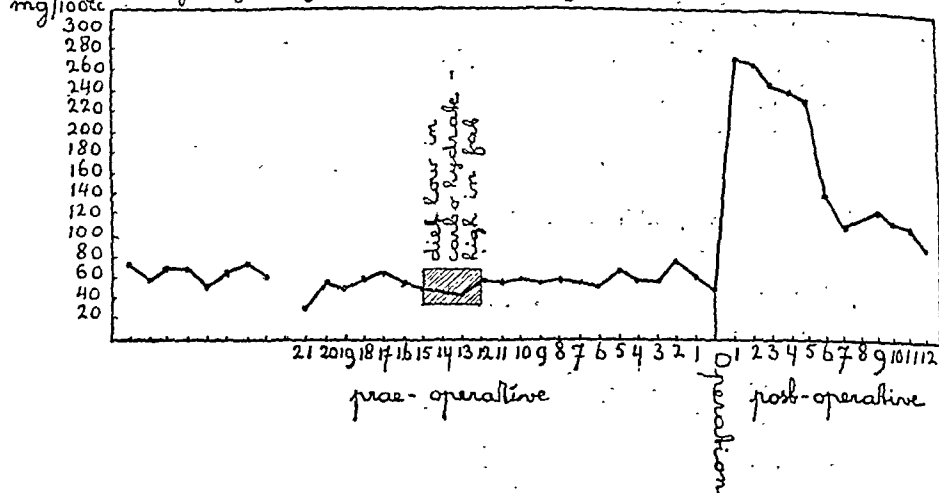
It is noteworthy that periods of higher and lower fasting blood-sugar values alternated: those observed in the Psychiatric Clinic, for instance, were higher than those afterwards found in St. Anthony Hospital (cf. Fig. I). The determination method followed in the Psychiatric Clinic is that of Hagedorn-Jensen, whereas in St. Anthony-Hospital that of Folin-Wu is used. As the results of the latter tend to be somewhat higher than those of the former, the differences between these two periods are in reality still larger.

The mild nature of the disease during the first years may have been due to the structure of the tumors. Whereas most of the adenomas, on account of the rich vascularisation, are red or purple,

Chart I

Blood sugar levels, fasting

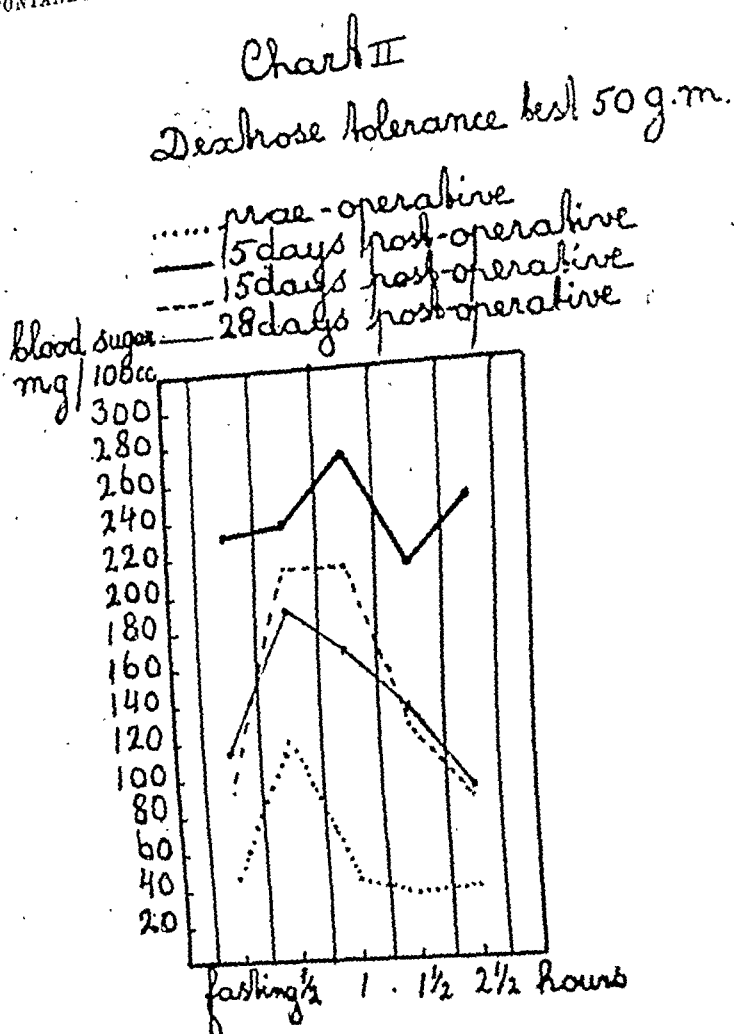
Blood sugar curve for Bigdeahy — St. Anthony Hospital



those found by us were of the same colour as the rest of the pancreas. The microscopical examination, moreover, proved that they contained much connective tissue. It is not impossible that for years but little insulin was given off by these adenomas to the circulation, which would explain the small number of attacks.

In the literature more of such protracted mild cases are to be found. From our own experience we infer that hyperinsulinism caused by adenomas such as ours may sometimes have been mistaken for functional hyperinsulinism.

Postoperatively a diabetes manifested itself, which lasted for 11 days (Fig. II). During the first 40 hours after the operation every 2—4 hours blood-sugar determinations were made and values varying between 0.276 and 0.206 per cent were found. During the first 24 hours the urine contained $2\frac{1}{2}$ per cent sugar. Four days after the operation the blood-sugar values were: fasting 0.228, after $\frac{1}{2}$ hour 0.234, 1 hour 0.254, $1\frac{1}{2}$ hours 0.212, $2\frac{1}{2}$ hours 0.246 per cent. From the fifth to the eleventh day after the operation the fasting value gradually sank from 0.140 to 0.112 per cent and on the twelfth day it was 0.092 per cent. On the fifteenth day the bloodsugar curve was: 0.094, 0.212, 0.214, 0.130, 0.084, per cent and on the twenty-eighth the values were: 0.116, 0.192, 0.168, 0.136, 0.081 per cent.



How is this post-operative diabetes to be explained?

As it lasted too long to be ascribed to manipulations carried out during the operation, two further possibilities remain:

1. It might be that the surplus of insulin had induced the production of a compensatory amount of insulin antagonists in the hypophysis. These antagonists remained in action for some time after the adenomas had been removed, but gradually the hormonal action returned to its normal level.

2. It might also be that the insulin requirements of the body were covered by the output of the adenoma and that the normal islet tissue had become inactive.

As the diabetes disappeared within a fortnight after the operation, the last supposition appears to us the more acceptable one.

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Protective value of the intracutaneous and percutaneous methods of BCG vaccination.

(A comparative experimental investigation).

By

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There are few subjects in medicine which have inspired such bulky and divergent views as Calmette and Guérin's BCG vaccine. This confusion has made clinicians adopt a reluctant »wait-and-see« attitude as long as their research-colleagues have not definitely established its merits as a safe and effective anti-tuberculosis measure. This traditional and highly commendable attitude has gradually changed into acquiescence in face of all the convincing experimental and clinical evidence in its favour. Thus, during the last five years, a universal interest has been aroused in its use as an important addition to existing anti-tuberculosis measures, even in Germany, where a law against its use was enacted shortly after the Lübeck tragedy in 1930.

Now that sufficient work has been done to prove that the BCG vaccine is harmless for animals and man, attention has become concentrated upon finding the ideal method for its administration to man. Desirable features of the ideal vaccination method would be the following: it should be relatively painless; it should produce a minimum of local blemish, and above all, it should give rise to a maximum of specific protection against attacks of virulent tubercle bacilli.

Specific protection against a virulent infection is produced only when the BCG vaccine enters the body parenterally and in turn is capable of starting certain cellular reactions which change the host into a medium unfavourable for the growth and spread of virulent tubercle bacilli.

The BCG vaccine stimulates a variable measure of such specific protection. It is unfortunate indeed that no biological test is available which discloses the degree of such acquired specific protection, except the experimental test of exposing the vaccinated animal to an inoculation with virulent tubercle bacilli. This is impracticable in man, although Calmette (1) expressed the opinion that in order to produce a permanent immunity, it was commendable to expose the relatively BCG-immunized individual to a virulent infection soon after vaccination. However, we know that the allergic state develops concurrently with the relatively immune state shortly after the BCG vaccination. Although those two states are independent and apparently antagonistic biological principles, it is an experimental and clinical fact that *a tuberculosis resisting organism is simultaneously an allergic organism*. The tuberculin test discloses reliably the state of allergic hypersensitiveness. Hence, on the basis of a positive tuberculin test we may discern the presence of the relatively immune state produced by the BCG vaccination, of which allergy is the shadow but not the substance.

On the basis of the incidence of positive tuberculin reactions after the BCG vaccination, we will be in a position to determine the ideal vaccination method which needs must be that which produces the greatest percentage of positive tuberculin reactions. If to this advantage we may also add the other desirable features of the ideal vaccine, namely, that its mode of administration produces a minimum of local blemish as well as a maximum of specific protection, then we shall readily hit upon the BCG vaccination method *par excellence*.

The burden of this present comparative investigation is to make a cursory survey of the intracutaneous and percutaneous methods of BCG vaccination now in vogue and to correlate these findings with our own experimental and clinical experience.

Oral, subcutaneous and intracutaneous BCG vaccination.

The discoverers of the BCG vaccine, Calmette and Guérin (2) were convinced that the *oral* administration of BCG to young calves produced an effective protection against a virulent tuberculous infection. When Weill-Hallé and Turpin (3) were entrusted by Calmette in 1921 to perform the first BCG vaccination on infants, they likewise administered the vaccine orally in 10 mg dosages on 3 successive days. Three months later only 2.5 percent of these infants had become tuberculin positive and 7.6 percent after 1 year. They presumed therefore that the orally administered BCG was mostly excreted with the faeces. This presumption was established experimentally on guinea pigs by Birkhaug (4). Weill-Hallé and Turpin abandoned thenceforth the oral method and began to inject the vaccine *subcutaneously* in 1924. The initial dosage of 0.25 mg. BCG produced such extensive local necrosis that the dosage was reduced to 0.01 and 0.02 mg. Now they obtained more than 90 percent positive tuberculin reactions already 3 months after vaccination. The subcutaneous method was still handicapped by the production of 30 percent suppurating abscesses in the regional lymph nodes and these healed very slowly and always left an unseemly scar.

Wallgren (5) tried to mitigate this handicap by injecting infants *intracutaneously* with 0.01—0.05 mg BCG. With this decidedly improved technique he obtained 93 percent positive tuberculin reactions in 6—7 weeks after vaccination and almost always after 16 weeks. However, it necessitated re-vaccination of 23 percent of the infants and some of these several times. In the hands of Wallgren, the intracutaneous injection produced only occasionally local necrotic lesions inasmuch as he practised extreme care in getting the vaccine into the uppermost epidermal layers. However, other physicians have been less successful in this respect. In one of the completest investigations of the intracutaneous BCG vaccination on infants and children, Kereszturi, Rosenberg and Park (6) made use of 0.03—0.3 mg. BCG. These workers reported the occurrence of 88 percent necrotizing local lesions and 25 percent of suppurating abscesses in the regional lymph nodes. In spite of these severe reactions, they obtained only 87 percent positive tuber-

culin reactions. They boldly state that in order to obtain 100 percent allergy, it would be necessary to produce suppurating abscesses.

As supervisor of the BCG laboratory, financed by the Norwegian National Tuberculosis Association, which distributes the vaccine to the entire country, Birkhaug (7) has collated the returned (incomplete) reports of persons vaccinated intracutaneously with 0.01—0.05 mg BCG during 1938—1941. He finds 86.4 percent of 652 persons vaccinated in 1938 became tuberculin positive approximately 3 months after vaccination; 89 percent of 603 persons vaccinated in 1939; 79.6 percent of 864 persons in 1940; 83.5 percent of 4544 persons in 1941 and 90.8 percent of 6256 persons vaccinated in 1942. During this period the reports show that suppurating abscesses varied between 23.6 percent in 1939 and 10.4 percent in 1942.

At the Municipal Tuberculosis Polyclinic at Oslo, Dahl (8) vaccinated 525 persons intracutaneously with 0.05—0.15 mg BCG in 1939 and obtained 95 percent positive tuberculin reactions (Pirquet). It should be mentioned that an induration measuring 2 mm in diameter 2 days later was considered as a positive test by Dahl while the National BCG Committee requires a minimum infiltration of 4 mm as a positive Pirquet test and 10 mm infiltration as a positive Mantoux test with 1 mg old tuberculin. This low limit must account for the high percentage of positive tuberculin tests obtained by Dahl. In regards to the frequency of suppurating abscesses, he writes (author's translation): «The blemishing abscesses in the vaccinated area was a most annoying complication during the early use of the intracutaneous vaccination method. But by cutting down the dose of vaccine from 0.10—0.15 mg to 0.05 mg BCG, the frequency of these abscesses was reduced from 39 to 6 percent.» In defending the intracutaneous versus the percutaneous BCG vaccination methods, he writes (author's translation): «Inasmuch as we mostly undertake mass-vaccination, it is essential to make use of a method which is effective and inoffensive, but also the least time-consuming. The prick with the needle and the burning pain caused by the injection, are not great inconveniences. But even so, they are doubtlessly more annoying than the pain inflicted by either Weill-Hallé's scarification or Rosenthal's multiple puncture methods. However, both these percutaneous methods require more

Courmont and Lesieur's observations stimulated other physicians to utilize the percutaneous route in attempts to prevent or to treat various forms of tuberculous disease. Cornell (17) claimed that inoculation with lupus built up an effective resistance against the disease. Maragliano (18) repeated this experiment on children by scarifying the arm through drops of heat-killed tubercle bacilli suspended in glycerine. Petruschky (19) maintained that good therapeutic results were obtained by rubbing an ointment of killed tubercle-bacilli into the skin in tuberculous patients. In a similar attempt, Moro (20) tried to facilitate the percutaneous absorption of the specific immunizing elements by adding a keratolytic substance to a tuberculin-salve containing equal parts of heat-killed bovine and human tubercle bacilli. More recently, Kutschera von Aichbergen (21) essayed the more drastic therapeutic measure of rubbing virulent tubercle bacilli into the scarified skin in patients suffering with pulmonary tuberculosis. For that very purpose, Coulaud and Mlle Lécuyer (22) used inunctions with heat-killed tubercle bacilli.

Decades before the above-mentioned experimental investigations, clinicians had made important observations on the rôle played by the skin in the production of anti-tuberculosis immunity. Already in 1886, Marfan (23) had formulated the law which bears his name, namely that: the healing of scrofula having taken place before the age of 15 years, confers a relative immunity against pulmonary tuberculosis. His classical words are as follows: *»Si on examine des adultes porteurs de cicatrices d'écrouelles, parmi ceux dont les adénites ont complètement guéri avant l'âge de quinze ans, on n'en trouve presque aucun qui soit atteint d'une tuberculose pulmonaire évolutive (guère plus de 1 sur 100). D'autre part, si on examine des sujets atteints de phtisie pulmonaire en activité, on en trouve fort peu qui présentent des cicatrices d'écrouelles complètement guéries avant l'âge de quinze ans (à peine 1 sur 100). Ce qui est vrai pour les écrouelles l'est aussi pour les autres affections scrofuleuses qui ont guéri avant quinze ans, à un degré un peu moindre toutefois.»* Marfan points out furthermore that lupus patients very rarely are affected by pulmonary tuberculosis. These basic facts are today universally recognized.

The relative anti-tuberculosis immunity against attacks of pulmonary tuberculosis, which obtains in lupous and scrofulous pati-

ents, makes Poinndorf and others (24) hypothesize that this immunity is produced because most of the manifestations of these diseases are referable to the skin. This supposition is succinctly worded by Marfan (25): *«D'après eux, la défense antituberculeuse de l'organisme serait surtout dévolue au tégument externe: c'est dans les cellules basales de l'épiderme que s'élaboreraient les substances antituberculeuses, mais elles ne les produiraient que lorsqu'elles sont en contact avec une lésion tuberculeuse. Un foyer bacillaire ne serait vaccinant que s'il est en contact avec la peau.»* Whatever truth is contained in this supposition, it must needs remain only a partial truth since it is well known that animals as well as man can be rendered allergic by non-tegmentous tuberculous infection. Nevertheless, they possess an undeniable antituberculous resistance without having at any time disclosed cutaneous manifestations of tuberculosis.

These examples of the ease with which tubercle bacilli can pass through the skin and produce disease and subsequently a considerable degree of anti-tuberculous resistance, should make better prototypes for Rosenthal's percutaneous multiple puncture BCG vaccination method than those references cited by him (12, 13, 14) in support of his unusually promising contribution to tuberculosis prophylaxis.

Rosenthal's percutaneous BCG vaccination method aims at the following advantages: «a) Easily applied so as to afford wide applicability. b) No gross local suppuration. c) No lymph-node suppuration.» Rosenthal's experimental work was done on 44 guinea pigs. He applied a drop of BCG vaccine containing 1.67 mg per cm³ to the epilated skin on each side of the vertebral column in the lumbo-sacral areas. The vaccine was spread across the skin and 30 firm tangential needle punctures were made on each side of the animal, making a total of 60 punctures. The vaccine was allowed to dry in 5 minutes and no bandage was applied to the vaccinated areas. Rosenthal writes that «no visible gross lesions appeared except for scabs produced at the scarified points. No visible scars resulted. The lymph nodes were not grossly palpable but were found enlarged when dissected out.» These observations of Rosenthal are faulty inasmuch as typical papular lesions are produced in the guinea pig as well as in man, as we shall presently describe in our own material. Microscopically Rosenthal found «typical tubercles developed at the junction of the epidermis and cutis and in the

cutis proper. The lymphatics contained a few polymorphonuclear leucocytes which had phagocytosed tubercle bacilli. Following the large single intracutaneous dose, the lymphatics were widely dilated and stuffed with cells and bacteria, amounting to a partial obstruction.» Twenty-four of the vaccinated animals were tested intracutaneously with 20 mg old tuberculin and later with 10 mg. Rosenthal found that 58.3 percent became positive in 1 week, 70.6 percent in 4 weeks, 90.9 percent in 6 weeks and 95.2 percent in 3 months. Nine months after the percutaneous vaccination 100 percent were tuberculin positive and they remained so for over 2 years.

In order to determine the rapidity with which the BCG vaccine was absorbed into the lymphatics and thence transported centripetally, Rosenthal excised the punctured sites, including the subcutaneous tissue and fat, at intervals from 5 minutes to 10 days after the percutaneous vaccination. By testing these animals afterwards with tuberculin, he observed that when the punctured skin was excised 2 hours after vaccination, the animal became tuberculin positive 3 months later. But when the skin was excised as late as 24 hours after vaccination, the animals became tuberculin positive 1 week later.

Unfortunately, Rosenthal did not complete his experimental investigation of the percutaneous BCG vaccination method by making protection tests for the purpose of evaluating the degree of acquired resistance toward an inoculation with virulent tubercle bacilli. Instead he transferred the study to newly-born infants at the Cook County Hospital in Chicago. On 133 infants he made 35 tangential needle punctures on the lateral aspects of the left arm through a drop of BCG which now contained 5 mg per cm³. Vaccination was performed on the fifth to seventh day of life. Again he makes a most inadequate description of the local reaction: »No visible local reaction of definitely palpable lymph nodes were noted. Pinpoint to pinhead sized scars were at times seen after three months.» He attempted to control the percutaneous BCG vaccination with a similar series of intracutaneous vaccinations with 0.038 mg BCG on the left arm. He describes that »a local nodule (5 to 8 mm) formed after three to five weeks which was either evacuated or completely absorbed. Lymph nodes were occasionally palpable. There was no general reaction in either method of vaccination.»

Eight days after delivery, or in other words 1 to 3 days after vaccination, the infants were returned home with their mothers. They were subsequently returned to the clinic after 3 months and next time after 12 months. This accounts for the inadequate descriptions of the local reactions. During these 2 visits, the vaccinated children plus a group of 232 non-vaccinated infants, were tested intracutaneously with 0.1 mg old tuberculin 3 months after vaccination and with 0.2 mg old tuberculin 12 months after vaccination. Identical positive 99—100 percent reactions were obtained in both the vaccinated groups while only 2.2 percent of the controls reacted positively (active tuberculosis). The incidence of suppurating lesions following the intracutaneous BCG vaccination is not stated, but Rosenthal remarks: »The mothers were apprehensive of the suppurating lesion that occurs after the single intracutaneous method of inoculation, but there was no systemic effect accompanying this lesion.» Rosenthal concludes from this not very profound study that »because the multiple puncture method gives equally as good results as a single intracutaneous injection it is felt that the former is superior because by this method there is (a) no gross local lesion, (b) no suppuration of lymph nodes, (c) no appreciable scar, (d) ease in administration and thus wider applicability, and (e) smaller dose of vaccine.

Nègre and Bretey (26) after learning of Rosenthal's work, immediately set about to determine the degree of tuberculo-resistance which the multiple puncture produced in the guinea pig. At the same time they modified Rosenthal's technique by making 5—6 scars of 1 cm length through dops of BCG containing 5 mg per cm. They observed that 47 percent of the multiple puncture group and 40 percent of the scarified group became tuberculin positive with 5 mg old tuberculin already 1 week after vaccination and 63 and 78 percent respectively after 1 month. By increasing the punctures from 30 to 60, they obtained 85.7 percent positive reactions after 1 month with 5 mg old tuberculin. The animals which had become tuberculin positive were now injected subcutaneously with 0.002 mg of a highly virulent human tubercle bacillus. Both the animals vaccinated by the multiple puncture and scarification methods presented a very marked protracted evolution of tuberculous lesions in comparison with the rapidly generalized tuberculosis in the controls. They concluded that »Il ressort de ces expériences que chez le

cobaye, aussi bien au point de vue de la sensibilisation que de la pré-munition, la méthode des piqûres de Rosenthal et celle des scarifications paraissent être aussi efficaces que les procédés de vaccination par les voies sous-cutanées ou intradermique habituellement employés.» But this conclusion is not based upon any simultaneously conducted investigation of these 4 methods, with a subsequent quantitative evaluation of the degree of tuberculosis in each of these groups and compared with non-vaccinated controls, which we shall attempt presently.

In a subsequent brief study, Nègre and Bretey (27) reported that when guinea pigs were scarified with 6 scars of 1 cm length through drops containing 5 mg BCG per cm^3 10 percent became tuberculin positive in 4—5 days and 90 percent in 20 days. When these vaccinated animals were injected subcutaneously with 0.001 mg highly virulent human tubercle bacilli, they found that their anti-tuberculosis resistance manifested itself already on the ninth day after vaccination. On the nineteenth day their resistance proved comparable with that usually observed 30—45 days after the intracutaneous vaccination. They hastened to conclude that: *«Il est donc permis d'espérer que si cette méthode est appliquée à l'enfant, elle aura entre autres avantages celui de réduire d'une façon appréciable la durée de son isolement après la vaccination par le BCG.»*

Weill-Hallé (28) who had innovated the subcutaneous BCG vaccination in infants in 1924 and regretted its high frequency of suppurating lesions, made immediate use of the percutaneous method on 40 infants. Finding the Rosenthal multiple puncture *«tatouage un peu pénible»*, he made use of the scarification method of Nègre and Bretey. Through drops of BCG (5 mg per cm^3) he made 2—3 crosses of 1—2 cm lengths on the lateral aspects of the arm. The scars were covered with gauze moistened with BCG and this was again covered with a rubber pad. This bandage was removed two hours later. A slight infiltration appeared in the scars in the course of 15—20 days and accelerated positive tuberculin reactions took place as described by Nègre and Bretey. It was necessary, however, to revaccinate 30 times before all these children became tuberculin positive.

After having visited Rosenthal at the Cook County Hospital in Chicago in September 1939, to learn the multiple puncture technique, Birkhaug (29) repeated Rosenthal's experimental work and

extended this in a series of 48 guinea pigs. He made use of 40 tangential punctures on each side of the vertebral column in the epilated lumbo-sacral areas through an emulsion containing 5 mg BCG per cm^3 . Instead of making the multiple punctures with one single needle as Rosenthal and Nègre and Bretey have done, Birkhaug improvised an apparatus in the shape of a rake bearing 8 gramophone needles with 3 mm interspace. The 40 punctures could thus be made rapidly with 5 downward strokes into the epidermis, leaving 3—4 mm space between each line of 8 punctures. The vaccine was allowed to dry in 5 minutes while the skin was held stretched. No bandage was applied to the vaccinated area. Three days later, he observed tiny red papules measuring in width 0.5—1 mm. In 1 week the papules measured 2—3 mm in diameter and their center was elevated 1—2 mm. In 3 weeks the redness had deepened and the underlying skin presented a palpable infiltration of approximately 5 mm thickness when the skin was folded together. Forty days after vaccination desquamation was in full progress and in some animals already completed. Thereafter, the skin became supple, flat, slightly rough and pigmented a delicate brawny colour in the punctured sites. The skin remained thickened 3—4 months after vaccination and the axillary lymph nodes which showed regularly a palpable hypertrophy 1 month after vaccination remained definitely enlarged up till 5—6 months after vaccination. The inguinal lymph nodes were on the other hand irregularly hypertrophied. The Mantoux test with 10 mg old tuberculin became positive (10 mm or more infiltration after 48 hours) in 58.3 percent in 1 week, 66.7 percent in 2 weeks, 83.3 percent in 3 weeks and in 100 percent in 4 weeks and until 14 months after vaccination when it began to wane.

Birkhaug determined the rate of dispersion of BCG from the punctures by excising the skin with its adjoining subcutaneous tissue and fat at intervals from 30 minutes to 20 days after vaccination and by testing the animals with 10 mg old tuberculin. He found that sufficient BCG had passed beyond the excised skin area within 2 hours after vaccination to render the animal tuberculin positive 4 months later. If excision was postponed until 24 hours after vaccination, the animal became allergic in 2—3 weeks. If delayed until 48 hours, the animal became invariably tuberculin positive 1 week later. These findings confirm in the main those

reported by Nègre and Bretey. The excised skin showed microscopically a moderate invasion of polymorphonuclear leucocytes and histiocytes into the papillary layer about 6 hours after vaccination as well as active phagocytosis in the various layers down to cutis proper. Within 10 days typical tubercles with numerous Langhans giant cells had formed in the papillary and reticular layers and after 20 days small nodules had formed which manifested no tendency to necrosis nor suppuration. Subsequent sections showed rapid resorption and connective tissue infiltration, scattered among which appeared nests of epithelioid cells and occasional giant cells. The same histopathological picture prevailed in the axillary, inguinal, periportal, retro-renal and tracheo-bronchial lymph nodes, with occasional acid-fast bacilli as late as 6 months after vaccination.

In order to determine the degree of tuberculo-resistance produced by the multiple puncture vaccination, Birkhaug superinfected 12 tuberculin positive guinea pigs with 0.001 mg of a highly virulent human tubercle bacillus simultaneously with 12 non-vaccinated guinea pigs. Eight weeks later all these animals were killed and their tuberculous lesions were compared quantitatively according to the weight and volume of various organs and lymph nodes. The control animals presented advanced caseo-necrotic lesions in the inoculated left thigh and adjacent lymph nodes, numerous tubercles in the spleen and marked hypertrophy of this organ, and scattered tubercles in the liver and the lungs. The vaccinated animals presented a small infiltrated area in the inoculated left thigh without any abscess, slightly enlarged adjacent lymph nodes and no macroscopic lesions in the internal organs or lymph nodes.

In another group of 12 guinea pigs immunized by the multiple puncture method, Birkhaug attempted to determine the approximate time after vaccination when the acquired tuberculo-resistance became effective against a virulent superinfection. For this purpose he injected these vaccinated animals subcutaneously in the left thigh with 0.001 mg of a highly virulent human tubercle bacillus. One animal was thus injected on each of the following days after vaccination: 3, 6, 9, 12, 15, 18, 21, 25, 27, 30, 40 and 50. A similar group of guinea pigs which had received no vaccine, was injected in the same order with 0.001 mg of the same virulent orga-

nism. Thus we have two comparable animals for every time interval: one immunized and one control. Each pair of animals was killed exactly 8 weeks after the virulent inoculation. The autopsy material, which was submitted to a careful quantitative analysis, revealed that no protective immunity was produced 9 days after the percutaneous vaccination, that a moderate immunity had developed 18 days after vaccination and that an effective resistance had been produced 21 days after vaccination. After this date, no macroscopic tuberculous lesions occurred in the internal organs and the virulent superinfection was sharply delimited to hypertrophied lymph nodes adjacent to the inoculated area in the left thigh. These findings were also a confirmation of the study by Nègre and Bretey.

Birkhaug was now encouraged to transfer the multiple puncture BCG vaccination to man. A group of 246 adults and 38 children were given 40 punctures with the above-mentioned 8-needled apparatus, on the lateral aspect of the arm. The vaccine emulsion contained 5 mg BCG per cm^3 . The punctured area was covered with a 4×4 cm sterile tissue paper moistened on the under surface with the BCG emulsion. With a firm grasp around the arm, the punctured skin area was stretched slightly in order to allow greater penetration of the vaccine through the dilated perforations in the epidermis. The paper was removed after 5 minutes. The papules developed in the same manner as described in the guinea pigs. The initial traumatic swelling and diffuse redness in the punctured sites gradually subsided after 3—5 days only to re-appear as intensely red papules during the second week after vaccination. The papules became elevated and indurated during the third week and measured from 2 to 4 mm in diameter and 1—2 mm in height. In the course of 4 to 7 weeks, the papules began to desquamate and gradually disappeared without having shown any tendency to ulceration. Usually the skin had regained its normal appearance two to three months after vaccination, with an occasional pinhead-sized brawny pigmentation in the punctured sites. The skin in the vaccinated area was definitely infiltrated during the entire papular phase and the nodular formations were hard when pressed between the fingers. After the visible healing and resorption of the papules, the skin remained slightly thickened and rough for as long as 3—6 months after vaccination. Occasion-

ally, the axillary lymph nodes on the vaccinated side were tender and palpably enlarged. No complaints of any general indisposition were made on account of the vaccination. When occasionally an allergic individual was vaccinated by mistake or during the pre-allergic phase of an incipient hilum adenitis or pulmonary tuberculosis, we observed already the following two-three days intensely red and markedly elevated 3—5 mm wide papules which rapidly became confluent to produce an inflamed and indurated area which measured in excess of 30×50 mm. The papules invariably pustulated in the centre within 10—16 days after vaccination, forming concave centers which healed by cicatrization. Usually these sites left pock-marks with ochre-pigmented centers. The axillary lymph nodes on the vaccinated side became invariably tender and markedly hypertrophied. The whole arm felt malaised and the constitutional symptoms of intoxication could be marked at the height of suppuration. The simultaneous occurrence of erythema nodosum in two adult individuals might have been provoked by the multiple puncture vaccination of allergic individuals. Needless to state that caution should be exercised in not admitting tuberculin positive individuals to BCG vaccination.

When the multiple puncture BCG vaccinated persons were tested with 1 mg old tuberculin intracutaneously 2 weeks after vaccination, 93.7 percent had already become tuberculin positive (10×10 mm infiltration or more in 48 hours). Four weeks after a successful multiple puncture vaccination (visible papules) all the vaccinated persons had become tuberculin positive. The tuberculin reaction with 1 mg old tuberculin continued to remain positive for 1 year and more. But inasmuch as no isolation were practised on the vaccinated persons, one cannot presuppose that the positive tuberculin reactions were wholly due to the percutaneous BCG vaccination. The results of our first multiple puncture vaccinations were so encouraging during 1939—1940 that all the subsequent BCG vaccination in Bergen were made with this method and shall be discussed presently.

This cursory review of the BCG vaccination methods practised since 1921 have made it clear that the Rosenthal percutaneous BCG method commends itself as the anti-tuberculosis vaccination method *par excellence*. Its double advantage is that it caters to the public demand for a painless and non-blemishing mode of vaccina-

tion and it satisfies the requirements of the research-worker and the hygienist of a relatively effective and widely applicable method, for preventing tuberculous disease.

The following comparative investigation of the intracutaneous scarification and multiple puncture BCG vaccination methods is undertaken at the urgent request of the Norwegian Physicians Tuberculosis Association's general assembly at Oslo in 1940.

Intracutaneous, scarification, and multiple puncture immunization with BCG.

Experimental data.

Animals. — Forty-eight normal albino guinea pigs, which failed to react with 10 mg old tuberculin injected intracutaneously, were divided into 4 equal groups, each group containing 6 male and 6 female animals. Group I was immunized by the multiple puncture method, group II by the scarification method, group III by the intracutaneous method and group IV was not immunized, but retained as controls for the virulent test infection.

Preparatory to the immunization process, the complete blood picture, with differential counts, was done on all the animals on 3 occasions with weekly intervals. The blood-work was continued with bi-weekly intervals throughout the entire experiment. The haematological findings will be published separately.

Chart I reveals that the average body weight for each group of animals was nearly identical when the experiment began and varied between 454 and 485 gms.

The animals were kept in groups of 6, of the same sex, in metal cages (46 × 46 × 35 cm) having closed sides and topped with a metalframed wire-lid with 3 × 3 cm wide meshes. The daily diet consisted of approximately 50 gms turnips, 75 gms hay, and 20 gms brown bread. Oats were not available and the brown bread was of inferior war quality. A glass pipette, placed through the lid, delivered water from an inverted bottle. Thrice weekly, cod-liver oil and »Vitakalk» were strewn on the slice of bread. The temperature of the animal house varied constantly between 15–18° C and ventilation was maintained by an electric suction fan. Inter-

current infection was absent from the animal house during the entire period of this experiment.

Immunization. — The animals in groups I, II and III about to be immunized were epilated in the right lumbo-sacral area with a paste made up of equal parts of barium sulphite and wheat flour mixed with water. The skin was afterwards thoroughly washed with lukewarm water. Vaccination took place the following day. The BCG vaccine (B—142) was freshly prepared from a 3 weeks old Sauton culture film and was made to contain 20 mg BCG per ml for the multiple puncture and scarification methods, and 0.5 mg per ml for the intracutaneous injection. These are identical with the concentrations of BCG employed on man.

The *multiple puncture* group of animals was vaccinated as follows: The animal was held tightly; the epilated skin was washed with 70 percent ethyl hydrate and then with ether. A few drops of the 20 mg BCG emulsion were spread evenly over the defatted skin area with a long needle. Our 8-needled apparatus was now pressed tangentially against the skin and lifted vertically with swift upward movement. The epidermis was thus torn open where the 8 needles had attached themselves to the skin. This process was repeated until 5 lines had been made with a total of 40 punctures. An interspace of 3—4 mm was allowed between each line. A few drops of the same BCG emulsion were spread over the punctured sites and a sterile tissue paper covered this area in order to retain the moisture in approximately 5 minutes. During this time the skin was stretched slightly in order to facilitate further penetration of the BCG vaccine. Five minutes later the paper was removed and no extra bandage was applied to the vaccinated area.

The *scarified* group of animals was prepared in the same manner except that 5 lines of approximately 4 cm's length and 3—5 cm apart were made with a vaccination lancet pressed hard against the skin, in the manner of the Pirquet tuberculin test.

The *intracutaneous* group of animals was injected with 0.1 ml of the BCG emulsion containing 0.5 mg per ml. The injection was made in the uppermost layer of the epidermis so as to form an elevated papule in the middle of the epilated area.

Vaccination reactions. — Already 3—5 days after the percutaneous vaccinations, 1.0 to 1.5 mm wide red points or streaks were visible. During the following week the inflammatory reactions

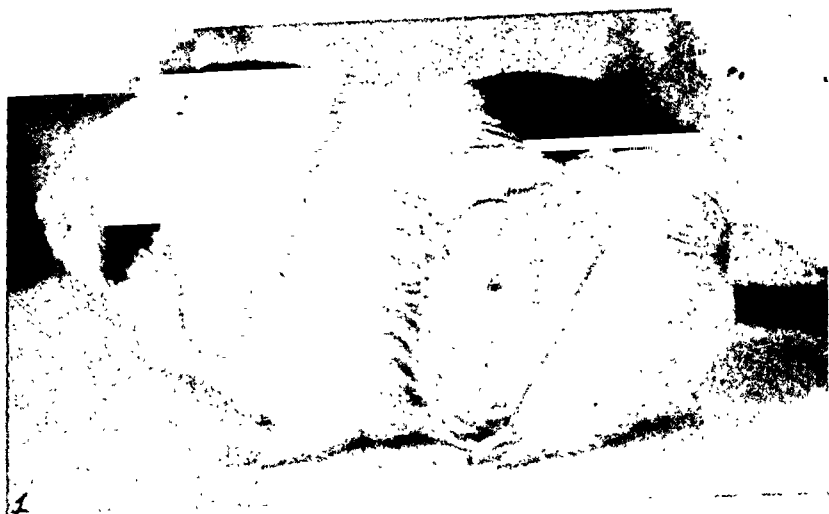


Figure 1. Showing the superficial abscess in the intracutaneously BCG vaccinated guinea pig 3 weeks after vaccination.

increased until the papules or scars measured 2—4 mm in width and 1—2 mm in height and the skin became palpably indurated. The axillary glands on the vaccinated side became slightly enlarged while the adjacent inguinal glands were not abnormally disturbed. Approximately 3 weeks after the percutaneous vaccination, the inflammatory reaction began to fade in the scarified animals while it persisted in the punctured animals. During the fourth week the healing processes were in full development and desquamation followed with the shedding of the crust. No suppuration took place in any of the percutaneously vaccinated animals. The underlying tissue remained considerably thickened and the adjoining axillary glands were further enlarged to pea-size, especially in the multiple puncture group while the inguinal glands had also palpably enlarged. Complete resorption and healing of the papules and scars occurred approximately 40 days after vaccination. At this time the skin had again become supple, although slightly roughened. A tawny pigmentation and slight depression indicated the vaccination sites. The axillary glands continued to remain palpably enlarged and firm as long as 3 to 5 months after vaccination while the inguinal glands had become reduced to normal size. The inflammatory reactions and the lymph nodes hypertrophy seemed definitely more intense in the multiple puncture group than in the scarification group. Figures 2 and 3 show the scars and punctures 3 weeks after vaccination.

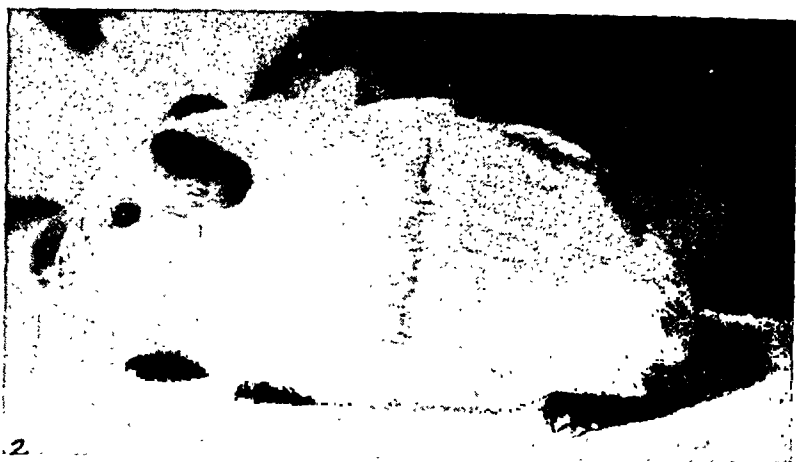


Figure 2. Showing the scarified area 3 weeks after vaccination.

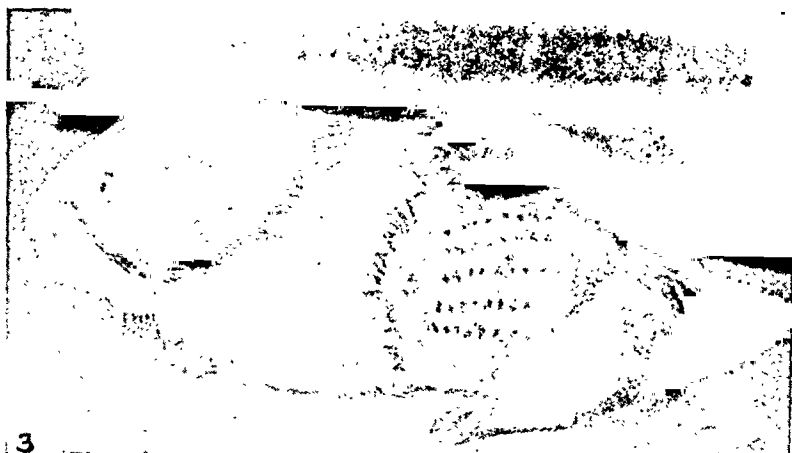


Figure 3. Showing the multiple puncture papules 3 weeks after vaccination.

The intracutaneously injected site became inflamed and elevated during the second week after vaccination and the ensuing cold abscess invariably perforated during the third and fourth week. Healing followed rapidly and the vaccination site presented a palpably infiltrated area having a concave center. The axillary glands were decidedly more hypertrophied during the fifth week than were the inguinal glands on the vaccinated side and the persistence of the glandular hypertrophy followed the same course as that described for the percutaneously vaccinated animals. Figure 1 shows the intracutaneous vaccination site 3 weeks after vaccination.

Post-vaccination tuberculin reactions. — The tuberculin tests were always made with 10 mg old tuberculin injected intracutaneously. The reading was made 48 hours afterwards and our minimum requirement for a positive reaction was an induration measuring crosswise 10 mm and when the skin was folded upon itself 4 mm. Half of this thickness multiplied by the 2 diagonal diameters of the indurated area should give us the approximate volume of the indurated area, namely $2 \times 10 \times 10 = 200 \text{ mm}^3$. It is regrettable that most experimental workers fail to state the actual measurements of the indurated area. Even as we demand definite measurements for a positive reaction in man, the same procedure should hold good for the experimental animal in order that one worker may compare his results with those of another. We have likewise assessed the approximate area of central necrosis within the tuberculin reaction by multiplying two diagonal measures with one another. Table 1 presents the data on the tuberculin reactions obtained during the third, sixth and eighth post-vaccination weeks in the 3 vaccinated groups of animals.

Table 1.

Tuberculin reactions with 10 mg tuberculin.

Post-vaccination weeks	Multiple Puncture			Scarification			Intracutaneous		
	%	Induration (mm ³)	Central necrosis (mm ²)	%	Induration (mm ³)	Central necrosis (mm ²)	%	Induration (mm ³)	Central necrosis (mm ²)
3	100	640	15.8	75	354	5.8	69	450	2.5
6	100	784	24.6	83	392	6.8	92	587	7.4
8	100	824	44.2	100	592	16.2	100	645	19.2

These figures reveal that the multiple puncture method produced the most rapid and intense tuberculin reactions, that the intracutaneous method held a close second while the scarification method lagged slightly behind.

Now that all the vaccinated animals had become allergic, we were ready to test their relative degree of acquired tuberculo-resistance by injecting them with a dose of virulent tubercle bacilli.

Virulent inoculation. — Fifty-eight days after the intracutaneous and percutaneous BCG vaccination, all the 36 immunized animals, plus 12 non-vaccinated control guinea pigs, were inoculated

intraperitoneally with 0.000.1 mg of a highly virulent strain of human tubercle bacilli («Tuxen»). This strain had been passed repeatedly through guinea pigs in order to sustain its virulence and the inoculating dose was prepared from a Löwenstein 4 weeks old culture of a guinea pig spleen. The inoculating dose was seeded out on a series of Löwenstein tubes which in the course of 10 weeks gave rise to 1283 eugonic colonies. We may infer therefore that each animal received approximately 1300 viable human tubercle bacilli.

We decided to make use of the intraperitoneal route in order to prevent the invariable suppurating lesion which results from the subcutaneous inoculation in the leg. Such infectious discharge entails a certain danger to the staff who handles the animals during the bi-weekly haematological work. It should be emphasized, therefore, that the intraperitoneal route of virulent infection presents a more immediate and greater tax on the protective mechanism in the vaccinated animals than does the subcutaneous inoculation which produces a slower progression of tuberculous lesions according to the law of Cohnheim.

Post-infection tuberculin reactions. — Eight weeks after the virulent inoculation, the tuberculin test was done on all the 4 groups of animals with 10 mg old tuberculin. Two days later we found that all the animals reacted positively. The control group presented an average induration which measured 2154 mm³ and central necrosis of 312 mm². The vaccinated groups presented less inflammatory reactions than the control group. Thus the multiple puncture animals showed an average induration of 1394 mm³ and central necrosis of 121 mm²; the scarification animals 1712 mm³ induration and 194 mm² central necrosis; the intracutaneous animals 1526 mm³ induration and 168 mm² central necrosis. If we suppose that the intensity of the tuberculin reaction mirrors the succession of events associated with tubercle formation through the successive stages of bacteriostasis brought about by the immunity acquired through BCG vaccination or progression because immune substances are lacking, then it would appear that the virulent infection proceeded most slowly in the multiple puncture group, a little less slowly in the intracutaneous group, still less in the scarification group and without any hindrance in the control group. This pre-supposition must needs await later verification.

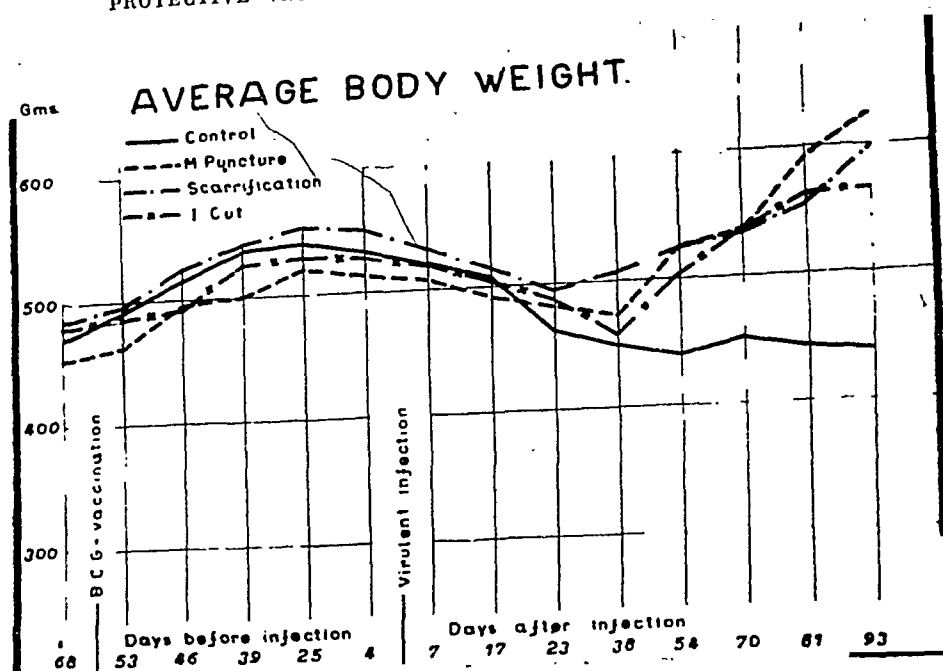


Chart 1. Average weight curves of 12 animals in each of the vaccinated and the control group during the entire experiment.

In order not to disturb the bi-weekly haematological studies, it was decided henceforth not to repeat the tuberculin tests on any group of animals.

Post-infection weight curves. — Chart 1 shows the average weight curves for the 4 groups of animals. These curves run a nearly parallel course until 1 month after the virulent infection. At this time the curve of the control animals begins a steadily downward course while those of the other 3 groups continue to rise sharply. Towards the end of the experiment we observe that the multiple puncture group leads with the highest average weight of 624 gms. The scarification group follows with 604 gms and the intracutaneous with 566 gms in contrast with 427 gms in the control group.

Mortality rate. — Two control animals died respectively 66 and 68 days after the virulent inoculation and presented generalized tuberculosis. Then followed 6 deaths with generalized tuberculosis among the same control animals, respectively 76 (1) 85 (1), 90 (2) and 102 (2) days after the virulent inoculation. Thus among a total of 12 control animals, 8 (66.7 percent) succumbed spontaneously with generalized tuberculosis before the experiment was terminated. In the scarification group 1 animal (8.3

percent) died 101 days after the virulent inoculation and in the intracutaneous group 2 animals died respectively 103 and 108 days after the virulent inoculation (16.7 percent). All of these immunized animals showed various degrees of generalized tuberculosis. During this period, none of the multiple puncture animals had died.

Macroscopic tuberculous involvement. — When 8 of the control animals and 3 of the immunized animals had succumbed spontaneously with generalized tuberculosis, it was decided to sacrifice all the surviving animals between 103 and 110 days after the virulent inoculation, in order to make a quantitative assessment of the degree of tuberculosis in each of the 4 groups of animals.

Table 2.

Macroscopic Scoring of Tuberculous Involvement in the Vaccinated and Control Animals.

(Ad modum S. A. Petroff and W. Steenken: J. Immunol., 1930, 19, 79).

Animal Groups	Number of animals	Tuberculous Involvement									
		++++		+++		++		+		0	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Control	12	10	83	2	17	0	0	0	0	0	0
Multiple Puncture	12	0	0	0	0	1	8	3	25	8	67
Scarification	12	0	0	3	25	5	42	4	33	0	0
Intracutaneous	12	1	8	3	25	4	33	2	17	2	17

0 = complete protection. + = tuberculous lesions at site of inoculation, with or without lymph node involvement, with a few tubercles in lungs and spleen. ++ = tubercles in 2 or 3 organs, such as spleen, liver, lungs and lymph nodes. +++ = generalized tuberculosis, as well as extensive active disease, such as tuberculous pneumonia. ++++ = fulminating and generalized tuberculosis, with multiple and large infarcts in spleen and liver; multiple cavernous and confluent tuberculosis in lungs, with heavy involvement of lymph nodes; ruptured spleen and allergic phenomena.

As on previous occasions, we delayed autopsy until the cadaver had remained in the ice-chest several hours in order to allow the blood to clot. Thus we obtained uniformity in the comparative assessment of the volume and weight of various organs.

Employing the scheme of Petroff and Steenken (30) for scoring of macroscopic tuberculous involvement in the viscera and lymph nodes among the vaccinated and control animals, we summarized these findings in Table 2.

It will be seen that 83 percent on the *control animals* presented a ++++ fulminating and generalized tuberculosis, with abscess in the abdominal wall through which the virulent inoculation took place, peritonitis, extreme fibrocaseous omental tuberculosis in which the omentum was rolled into a huge sausage form lying under the stomach and adhering to surrounding structures, multiple and large infarcts in the spleen and liver, multiple cavernous and confluent tubercles in the lungs, and heavy caseo-necrotic involvement of lymph nodes. The remaining 17 percent of the control animals presented a +++ generalized tuberculosis of slightly less involvement in the viscera than the aforementioned. In contrast with this extreme form of experimental tuberculosis, we find that 67 percent of the *multiple puncture animals* showed complete protection, without any macroscopic lesions whatsoever; 25 percent presented a + tuberculosis in lymph nodes and an occasional tubercle in the spleen or lungs. The remaining 8 percent of the multiple puncture animals presented a ++ tuberculous involvement in lymph nodes, the spleen, liver or lungs. The remarkable feature in this group of protected animals was the absence of any lesion in the abdominal wall and normal appearance of the omentum, except in the one animal which presented a ++ tuberculosis; here the omentum was found adherent to the abdominal wall and seeded with numerous small fibrous tubercles.

In the *scarification animals* none showed complete protection, 33 percent presented + tuberculous involvement in lymph nodes and an occasional tubercle in the spleen or lungs; 42 percent showed ++ tuberculous lesions 2 or 3 organs, while only 25 percent presented +++ generalized tuberculosis, involving the abdominal wall, omentum, peritoneum, spleen, liver, lungs and lymph nodes.

In the *intracutaneous animals* we encountered 17 percent completely protected; 17 percent presenting + and 33 percent ++ tuberculous involvement; 25 percent showed +++ generalized tuberculosis and 8 percent presented ++++ fulminating and generalized tuberculosis.

It is quite apparent from this macroscopic scoring that the immunity which various modes of BCG vaccination bestow on the guinea pig is not absolute, but nevertheless of a relatively considerable degree. It is not difficult to discern that the most effec-

tive protection obtained in the multiple puncture vaccinated animals and that the intracutaneously vaccinated animals reached a slightly higher protection than the scarification animals.

It should be needless to state, however, that the quantitative approach to the assessment of differences in tuberculous hyperplasia in these variously vaccinated groups of animals commends itself to the experimental worker rather than the less exact subjective description of the degree of tuberculous involvement in terms of one or more plusses. Lord Kelvin's remark is very apropos: »When you can measure what you are speaking about and express it in numbers, then you know something about it. But when you cannot measure it, nor express it in numbers, then your knowledge is of a meagre and unsatisfactory kind.»

Quantitative assessment of tuberculous hyperplasia.

The most exact method for determining the tuberculous hyperplastic changes which have taken place in viscera or lymph nodes, is by the water displacement apparatus described by us in 1940 (31). The smallest of these apparatus is calibrated down to 0.001 ml. The data in Tables 3 and 4 are collected by means of these apparatus. The weight of the spleen, liver, lungs and pooled (total) lymph nodes is known to vary according to the animal's body weight. We have therefore converted these weights in Table 4 into the more exact percent of the animal's body weight. The completed data are submitted to statistical analysis according to Fisher's (32) method for comparison of two comparable means (\bar{x} and \bar{y}).

In the present investigation we have chosen to reject as insignificant any mean value of P (probability) which is more than 0.01. The symbol $P \leq 0.01$ signifies, therefore, that the observed mean deviations bearing this or smaller values must be considered to have *absolute statistical significance* and cannot have occurred by chance alone (33). In Fisher's 1938 tables for distribution of t (quotient expressing the deviation as a multiple of its probable error), we find that a $P \leq 0.01$ requires that $t \geq 2.819$ when each group contains 12 animals or samples. Every difference having *absolute significance* is italicised in Tables 3 and 4. »Sigma» or the *standard deviation* (square root of the arithmetic mean of the squares of

the deviations from the average) will enable the reader to follow the actual dispersion from the average within the group.

Volume of lymph nodes. — The volumetric data on 16 different sets of lymph nodes and also on the omentum are presented in Table 3. At the extreme right we have placed the statistical analysis of these data in terms of the quotient of probable error (t) and degree of significance (P). A glance at Chart 2, in which we have drawn the average volume of the left and right superior inguinal, femoral and tracheo-bronchial lymph nodes as well as the periportal lymph node, makes at once clear that the smallest nodular hypertrophy has taken place in the multiple puncture immunized group; that the hypertrophy is decidedly greater in the lymph nodes taken from the intracutaneously immunized group and that those from the scarified group of immunized animals are slightly more hypertrophied than the nodes from the intracutaneous group. And yet, with the exception of the periportal node, the nodes from the control group are approximately twice the size of those taken from the intracutaneous and scarification groups. The protection offered by the BCG-vaccination is therefore remarkably high, but varies according to the mode of vaccination. The same is true for the data on the omentum, the organ which bears the immediate brunt of the intraperitoneal inoculation with virulent tubercle bacilli and where surviving organisms are quickly segregated by the rapid formation of tubercles. The omentum reflects the relative degree of immunity which each vaccinated group possesses. Thus, we observe that while the omentum from the multiple puncture group measures 0.89 ml, it measures 2.47 ml in the scarification group and 2.69 ml in the intracutaneous group and approximately 3 times as much (7.43 ml) in the control group.

Without going into details about the differences in average volumes of the lymph nodes and the omentum in the 4 group of animals, let it suffice that the differences in volumes of lymph nodes and the omentum in the multiple puncture and control groups (I vs IV) gave 100 percent significant results in favour of the multiple puncture method of immunization, 83 percent when the intracutaneous and control groups (III vs IV) were compared and 53 percent when the scarification and control groups (II vs IV) were compared.

Table 3.

Tuberculous Hyperplasia in Lymph Glands and in the Omentum.

Statistical analyses of volume (in ml) of lymph glands from vaccinated and control animals (12 animals in each group).

Lymph Nodes	I Multiple Puncture		II Scarification		III Intracuta- neous		IV Control		Probability					
									I vs IV		II vs IV		III vs VI	
	Av.	Sigma	Av.	Sigma	Av.	Sigma	Av.	Sigma	t	P	t	P	t	P
Krbe Lf.	0.01	0.003	0.04	0.009	0.03	0.009	0.17	0.088	4.900	< 0.001	3.883	< 0.001	4.182	< 0.001
» Rt.	0.01	0	0.04	0.045	0.03	0.020	0.15	0.085	5.042	< 0.001	3.545	< 0.01	4.082	< 0.001
Sup. inguinal Lf.	0.12	0.050	0.40	0.251	0.30	0.176	0.82	0.296	5.772	< 0.001	2.678	0.02	3.692	< 0.01
» » Rt.	0.10	0.096	0.32	0.182	0.31	0.170	0.80	0.296	6.081	< 0.001	3.530	< 0.01	3.798	< 0.001
Deep » Lf.	0.02	0.010	0.07	0.042	0.03	0.017	0.17	0.092	3.949	< 0.001	2.450	0.02	3.648	< 0.01
» » Rt.	0.01	0.001	0.08	0.056	0.02	0.009	0.16	0.066	4.711	< 0.001	2.107	0.05	4.341	< 0.001
Femoral Lf.	0.05	0.032	0.29	0.212	0.16	0.076	0.47	0.136	8.575	< 0.001	1.547	0.15	5.582	< 0.001
» Rt.	0.04	0.008	0.23	0.140	0.15	0.085	0.41	0.140	9.156	< 0.001	2.450	0.02	4.976	< 0.001
Trach. bronch. Lf.	0.22	0.128	0.81	0.293	0.68	0.335	1.20	0.460	5.723	< 0.001	1.999	0.05	2.531	0.02
» » Rt.	0.20	0.113	0.72	0.326	0.71	0.377	1.10	0.580	4.920	< 0.001	1.808	0.08	1.793	0.10
Cervical Lf.	0.09	0.045	0.30	0.180	0.20	0.069	0.42	0.186	3.740	< 0.001	0.948	0.35	2.450	0.03
» Rt.	0.09	0.051	0.18	0.074	0.16	0.053	0.42	0.147	5.424	< 0.001	3.768	< 0.001	4.246	< 0.001
Axilla Lf.	0.07	0.033	0.18	0.110	0.19	0.089	0.40	0.061	13.475	< 0.001	4.900	< 0.001	5.708	< 0.001
» Rt.	0.06	0.056	0.16	0.088	0.15	0.061	0.33	0.123	6.615	< 0.001	3.084	< 0.01	3.937	< 0.001
Periportal	0.48	0.178	1.32	0.507	1.10	0.523	2.07	0.450	8.131	< 0.001	2.754	< 0.01	3.626	< 0.01
Mesenteric	0.30	0.186	0.80	0.533	1.00	0.537	3.55	1.309	5.897	< 0.001	4.581	< 0.001	4.368	< 0.001
Omentum	0.89	0.240	2.47	1.000	2.69	0.956	7.43	1.916	9.155	< 0.001	5.032	< 0.001	5.895	< 0.001
Efficiencies in percent									100 %		53 %		83 %	

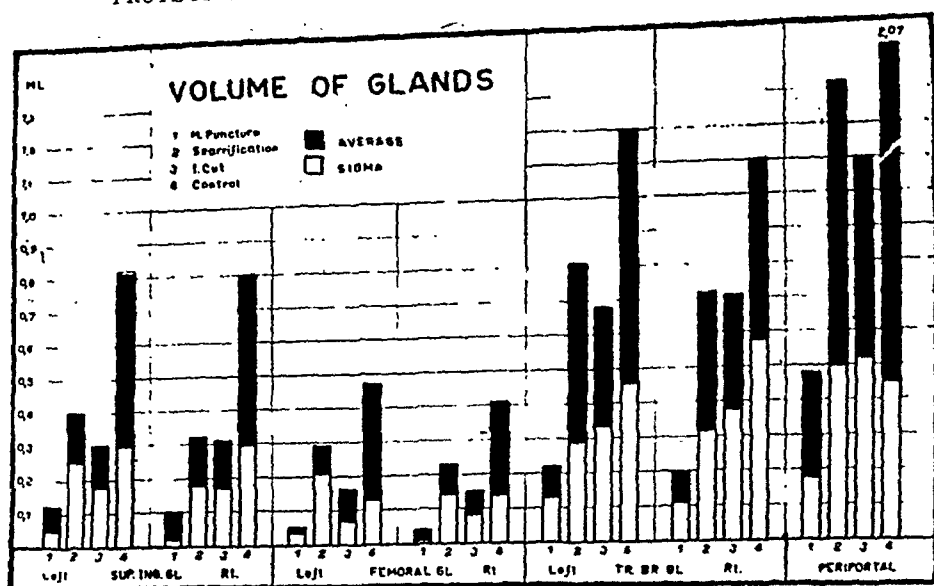


Chart 2. Average volumes (ml) of lf. and rt. superior inguinal, femoral, tracheobronchial and the periaortic lymph nodes in the vaccinated and control groups of animals.

Volume and weights of spleen, liver, lungs and lymph nodes. — Table 4 presents the data on the tuberculous hyperplasia in the viscera and lymph nodes in terms of grammes, ml, and percent of body weight. These data are summarized graphically in Chart 3. Again we observe the very relationships of protection in the 3 immunized groups as we saw in Chart 2 for the volumetric data on lymph nodes: the multiple puncture group presents the least hypertrophy as measured by volume, weight and percent of body weight; the scarification and intracutaneous groups approximate each other in all these 3 forms of measurements, while the control measures are excessively greater than any within the immunized groups. These striking immunizing effects of the 3 methods of BCG vaccination are reflected in the 100 percent significant deviations in every one of the immunized groups from the measurements in the control group.

If we should venture to fine-comb the quotients of probable error (t), in order to discern any superiority of significant deviations among the 3 groups of immunized animals, we would find that the average t is 6.540 when the multiple puncture deviations are compared with those in the control group (I vs IV); that it is 5.015 when the intracutaneous and control group deviations are

Table 4.

Tuberculous Hyperplasia in Spleen, Liver, Lungs, and Total Lymph Nodes.

Statistical analyses of their weights, volumes and weights expressed in percent of the animals's body weight (12 animals in each group).

Organs	I Multiple Puncture		II Scarification		III Intracuta- neous		IV Control		Probability					
									I vs IV		II vs IV		III vs IV	
	Av.	Sigma	Av.	Sigma	Av.	Sigma	Av.	Sigma	t	P	t	P	t	P
Spleen gms	0.90	0.308	1.60	0.441	1.50	0.583	5.10	2.208	4.875	< 0.001	4.042	< 0.001	4.101	< 0.001
Liver gms	35.3	2.691	35.6	5.700	35.8	7.600	46.8	7.333	4.013	< 0.001	3.325	< 0.01	3.016	< 0.01
» ml	34.5	3.291	34.0	5.830	33.8	5.691	47.6	7.383	4.469	< 0.001	3.998	< 0.001	3.981	< 0.001
Lungs gms	7.4	0.475	8.1	1.433	7.2	1.400	13.6	3.408	5.503	< 0.001	4.432	< 0.001	4.961	< 0.001
» ml	11.5	1.266	11.3	1.425	10.8	1.958	16.5	4.041	3.450	< 0.01	3.628	< 0.01	3.675	< 0.01
Lymph nodes gms	3.4	1.300	11.0	3.633	10.0	3.025	21.2	5.030	9.045	< 0.001	4.486	< 0.001	5.157	< 0.001
Weight of organs expressed in percent of body weight														
Spleen %	0.14	0.046	0.29	0.123	0.28	0.117	1.19	0.396	5.358	< 0.001	4.498	< 0.001	4.550	< 0.001
Liver %	5.69	0.483	5.96	0.721	6.37	0.604	10.90	1.341	9.249	< 0.001	5.500	< 0.001	7.548	< 0.001
Lungs %	1.20	0.121	1.43	0.398	1.35	0.440	3.81	0.733	8.881	< 0.001	6.941	< 0.001	7.441	< 0.001
Lymph nodes % ..	0.50	0.206	1.96	0.734	2.25	0.539	4.98	1.090	10.552	< 0.001	5.966	< 0.001	5.716	< 0.001
Efficiencies in percent							100 %		100 %		100 %		100 %	

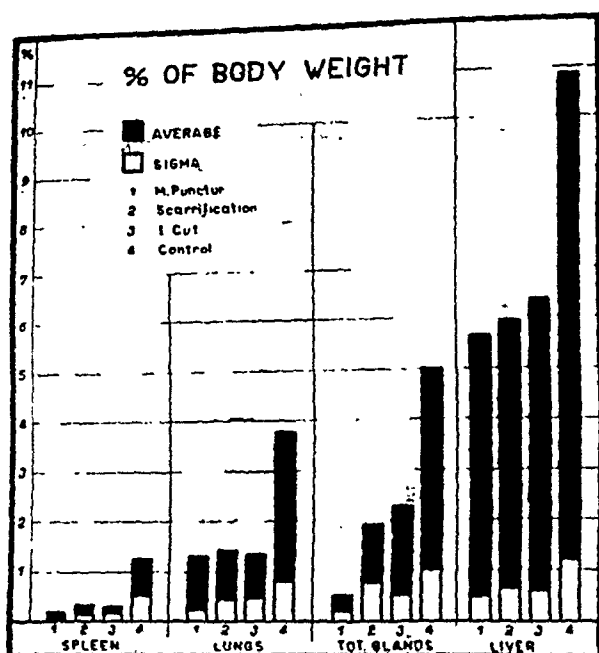


Chart 3. Average weight of spleen, liver, lungs and the pooled total lymph nodes, expressed as percent of the animal's body weight.

compared (III vs IV) and 4.682 when the scarification and control group deviations are compared (II vs IV). Hence, we may infer that the highest immunizing effect against a virulent infection is produced in the multiple puncture group and the least protection produced by the scarification method, although it lays only slightly behind the potency imparted by the intracutaneous method. But by and large, it should be emphasized that any one of the 3 methods of vaccination yields deviations from the unprotected control group which are larger than 3 times the probable error and as such must be accepted to be of an undisputed statistical significance (32).

In Table 5 we have attempted to discern to what degree the significant deviations in one vaccinated group differed from those in another vaccinated group in order to settle the question of the most effective mode of vaccination. Thus we find that when the multiple puncture deviations are placed against those in the scarification group, the former method yields 51.9 percent significant deviations from the latter. The same percentage figure holds good when the multiple puncture deviations are compared with the intracutaneous group deviations and again in favour of the

Table 5.

A Comparative Statistical Analysis of the Significant Deviations in one Vaccinated Group placed against those in another.

Organs	Multiple Puncture vs Scarification		Multiple Puncture vs Intracutaneous		Scarification vs Intracutaneous	
	t	P	t	P	t	P
Knee gld. lf.	3.567	< 0.01	2.565	0.02	0.888	0.40
» » rt.	2.255	0.03	2.197	0.04	0.622	0.50
Sup. inguin. gld. lf.	2.658	0.015	2.249	0.04	0.785	0.45
» » » rt.	2.881	< 0.01	3.317	< 0.01	0.104	0.90
Deep » » lf.	3.109	< 0.01	1.346	0.20	2.367	0.03
» » » rt.	3.369	< 0.01	2.076	0.05	2.820	< 0.01
Femoral gld. lf.	2.251	0.03	3.631	< 0.01	1.101	0.30
» » rt.	3.040	< 0.01	3.151	< 0.01	1.133	0.25
Trach. bronch. lf.	5.162	< 0.001	3.511	< 0.01	0.802	0.45
» » rt.	4.163	< 0.001	3.739	< 0.001	0.058	0.90
Cervical gld. lf.	2.237	0.03	3.650	< 0.01	1.042	0.30
» » rt.	2.940	< 0.01	2.734	0.02	0.632	0.55
Axilla. gld. lf.	2.668	0.015	3.719	< 0.001	0.204	0.85
» » rt.	2.634	0.015	3.939	< 0.001	0.240	0.80
Periportal gld.	4.378	< 0.001	3.374	< 0.01	0.883	0.40
Mesenteric gld.	1.998	0.06	3.543	< 0.01	0.651	0.50
Omentum	3.307	< 0.01	4.545	< 0.001	0.364	0.70
Spleen weight	3.430	< 0.01	2.489	0.03	0.394	0.70
» percent	2.783	< 0.01	3.063	< 0.01	0.152	0.90
Liver weight	0.134	0.90	0.190	0.85	0.063	0.90
» volume	0.208	0.85	0.281	0.75	0.066	0.90
» percent	0.848	0.40	2.006	0.06	1.057	0.30
Lungs weight	1.173	0.25	0.291	0.75	1.040	0.30
» volume	0.443	0.65	0.887	0.40	0.544	0.60
» percent	1.173	0.25	0.910	0.35	0.327	0.75
Total glds. wt.	5.909	< 0.001	6.010	< 0.001	0.631	0.50
» » percent.	5.086	< 0.001	7.350	< 0.001	0.804	0.40
Efficiency percent	51.9		51.9		3.7	

former method. On the other hand, we obtain only a 3.7 percent efficiency when the scarification group deviations are placed against those in the intracutaneous group. From this we may conclude that the 2 latter methods are nearly identical in their immunizing potencies while the multiple puncture method yields results slightly ahead of these 2 other methods.

Surviving tubercle bacilli in the spleen. — It will be noted in Table 4 that the volume of the spleen was not determined. This precaution was taken in order not unduly to contaminate the spleen which should be cultured for determination of viable tubercle bacilli in definite weights of the organ. The well-known predilection of the guinea pig spleen for tuberculous infection made Krause (33) state: »In guinea pigs... the spleen is undoubtedly the organ that is most prone to tubercle.»

The spleen was removed, weighed and homogenized under strictly aseptic conditions. Adventitious bacteria were destroyed by the usual procedure of emulsifying the splenic tissue with exactly measured 3 percent NaOH, incubation for 30 minutes at 37.5° C., centrifugalization in 30 minutes at 3500 r. p. m., decantation of supernatant fluid and neutralization of the residue with exactly measured 5 percent HCl in the presence of 3 drops of sterile 5 percent litmus. Having calculated all dilution factors, the resultant emulsion was diluted serially with saline until 0.5 ml contained 0.02 gm, 0.002 gm and 0.000.2 gm of the original splenic tissue. These amounts were delivered with Pasteur pipettes onto the slanted surface on 50 ml tubes of Löwenstein's solid egg medium containing 0.75 percent glycerin and 0.013 percent malachite green dye. The cotton-plugged tubes were left in horizontal position in the incubator until excessive liquid had evaporated when the tubes were sealed with rubber caps and left in the incubator for 10 weeks. The spleens of 8 animals from each group were cultured in this manner. The resultant eugonic colonies of the »Tuxen» strain of human tubercle bacilli employed for the virulent infection are supposed each to represent a single tubercle bacillus from which the colony has developed during 10 weeks (hypothetical). Table 6 tabulates the number of colonies found in each culture.

It will be observed that a direct relationship obtains between the degree of tuberculous involvement and number of viable tubercle bacilli found in the spleen. Thus we find that 0.3 bacillus was present in 0.002 gm of the spleen taken from 8 multiple puncture animals; 2.5 bacilli in the same weight of spleen in the intracutaneous animals and 3.5 bacilli in the scarification animals while the control animals presented 50.1 bacilli per 0.002 gm of the spleen. This crucial proof of effective bacteriostasis in every one of the BCG immunized groups of animals confirms in large the

Table 6.

Bacterial Cultures of Homogenized Spleen from Vaccinated and Control Animals.

Animals		Degree of tbc.	Colonies of tubercle bacilli in following weights of homogenized spleen		
Group	Nr.		0.02 gm.	0.002 gm.	0.000.2 gm.
M. Punct.	32—1	+	6	0	0
	» 3	+	7	0	0
	» 4	++	21	2	0
	» 6	0	4	0	0
	17—1	0	4	0	0
	» 2	0	1	0	0
	» 3	0	0	0	0
	» 6	0	1	0	0
Average			5.5	0.3	0
Scarif.	19—1	++	10	1	0
	» 2	++	8	0	0
	» 3	+++	82	8	0
	» 5	++	23	3	0
	» 6	+	1	0	0
	36—1	++	23	3	0
	» 3	+++	128	13	0
	» 4	+	4	0	0
Average			34.9	3.5	0
Intracut.	34—1	+++	44	4	0
	» —3	+++	34	3	0
	» —5	++	22	3	0
	21—1	+	5	0	0
	» 2	0	2	0	0
	» 3	++	22	2	0
	» 4	++	32	4	0
	» 5	++	27	3	0
Average			23.5	2.5	0
Control	14—1	++++	∞	112	15
	» 3	++++	∞	68	8
	» 4	++++	∞	24	3
	» 5	++++	∞	21	2
	» 6	++++	∞	78	9
	12—1	++++	∞	21	3
	» 4	++++	∞	33	3
	» —6	++++	∞	44	5
Average			∞	50.1	6.0

macroscopic, microscopic and quantitative observations already made on the tuberculous involvement in these 3 groups of animals and that the most effective bacteriostasis obtains in the group of animals vaccinated with the multiple puncture method.

The fact that not all the virulent tubercle bacilli were destroyed in the BCG vaccinated animals suggests the relative character of the acquired immunity. This agrees with our previous experience with iathergic (desensitized and immunized) animals. In this connexion it should be recalled that even in a disease such as vaccinia, which confers a solid immunity, the virus was concentrated by cataphoresis by Olitsky and Long (34) considerable time after immunity had been established. Other things being equal, it is most probable that the effective protection in our vaccinated animals would rapidly diminish when the primary BCG lesions had become completely resorbed. But by terminating the experiment at the time when the control and unprotected animals showed maximum tuberculous involvement, we were in position to determine accurately the relative degree of protection which the 3 modes of BCG vaccination had produced. The relative character of the experimentally acquired immunity in the guinea pig vaccinated with BCG obtains *pari passu* in man. Clinical experience has taught us that when the allergy produced by the BCG vaccination burns out in man, then his tuberculosis resistance falters and re-vaccination is necessary.

In a choice of vaccination method, our investigation gives preference to the multiple puncture method. But the all essential experimental fact remains, that any one of the parenteral methods employed in this investigation is capable of producing a significant tuberculo-resistance.

Multiple puncture method in man. — We have already referred to our very first clinical results with the multiple puncture vaccination on 246 adults and 38 children (29). Since then we have vaccinated in the period December 1, 1940—December 31, 1942, with the 8-needled puncture apparatus previously mentioned, 3727 children and adults at the City Public Health Bureau's Tuberculosis Polyclinic. On infants we practised 16 punctures; on children and women 24 punctures and on men 40 punctures. Of these, 3126 (84.1 percent) came to control study 4 to 8 weeks after vaccination and 3042 (97.3 percent) reacted with more than 10×10 mm

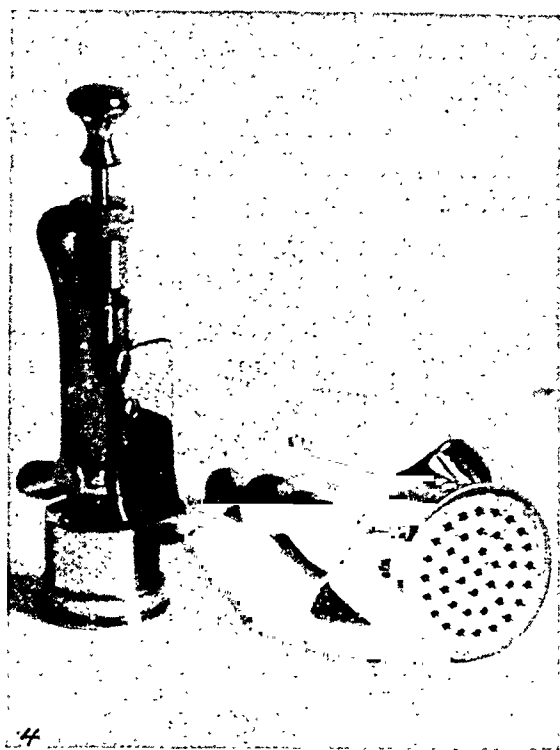


Figure 4. The automatic BCG multiple puncture apparatus. Height 12.5 cm and largest diameter 4.3 cm.

induration 48 hours after the intracutaneous injection of 1 mg old tuberculin. Only in 118 (3.8 percent) did we observe pus in the multiple papules and these healed promptly after application of cod-liver oil salve. Of the 2307 persons who were vaccinated by the multiple puncture method in the period December 1, 1940—April 4, 1942, there came to the yearly follow-up control 1285 (55.8 percent). When tested with 1 mg old tuberculin intracutaneously, 1188 (92.5 percent) reacted positive. Elevated or excavated scars were noticeable on 34 persons (2.7 percent) and small pin-head sized white marks were barely visible on close inspection on 26 persons (2.0 percent). No case of destructive tuberculous infection was found among this group of BCG vaccinated persons. A more detailed study will shortly be published on this group.

In order to facilitate the multiple puncture technique for the general practitioner, we found it convenient to produce an automatic BCG multiple puncture apparatus, built on the pistol-snap



Figure 5. The automatic BCG multiple puncture apparatus showing the 40 protruded needles.

principle. With the cock drawn up, a plate bearing 40 needles was lifted into a safety-box. By pressing on a button, the needles were released with some force and protruded from perforations in the bottom-plate of the apparatus. The depth of the needles was usually 2—3 mm. (Figs. 4 and 5).

The following technique has been found useful both for individual and mass vaccination: The skin on the lateral aspects of the arm is cleansed thoroughly with aether. A 4×4 cm steril piece of very thin tissue paper or celophane is moistened on both sides in the 20 mg per ml BCG vaccine in a sterile Petri dish and is placed on the aether-cleansed skin. The cock on the apparatus is drawn up, the end-plate is pressed against the paper while the button is pressed which releases the needle-plate. The vaccination is finished and the arm is held tightly in order to stretch the skin slightly. After 2 minutes the vaccinated person can leave without any bandage over the vaccinated area. By mass-vaccination, we have placed a series of 20—30 papers in tile-formation in the BCG-emulsion, leaving a 1—2 mm free edge which facilitates lifting the paper with a forceps. Figure 6 shows a typical «take» 3 weeks after vaccination. (36)

During 1943 we have vaccinated approximately 1500 persons with the automatic apparatus and have obtained 98 percent posi-

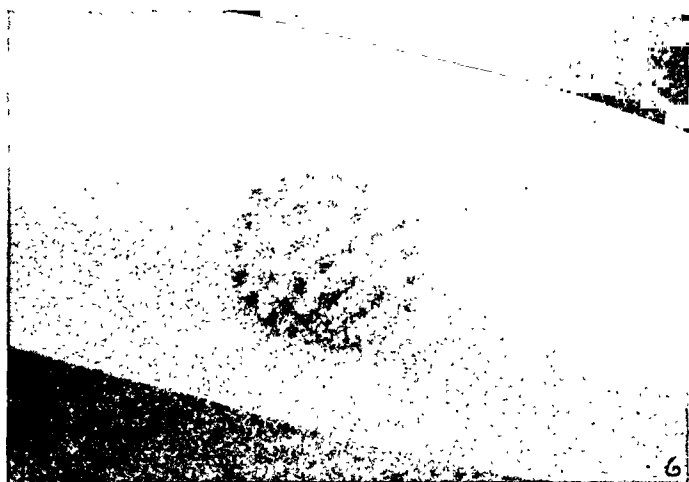


Figure 6. Showing the pattern of 40 papules 3 weeks after vaccination.

tive tuberculin reactions with 1 mg old tuberculin 2 months after vaccination. One important fact must be borne in mind with this technique and that is that the paper must be thoroughly soaked with the 20 mg per ml BCG emulsion. One physician can easily vaccinate 200 persons per hour when the assisting nurses make the proper arrangements. We shall shortly make public our detailed experience with the percutaneous method of BCG vaccination. Because of difficulties with obtaining the essential metals, we have only been able to make a limited number of these automatic apparati.

Conclusions.

BCG vaccination performed either by the intracutaneous or the percutaneous (multiple puncture or scarification) methods produces in the guinea pig a significant resistance against a virulent tuberculous infection.

The multiple puncture BCG vaccination method produces the most potent tuberculo-resistance in the guinea pig.

The multiple puncture BCG vaccination in nearly 5000 children and adults produced 98 percent positive tuberculin reactions with 1 mg old tuberculin in two months.

An automatic apparatus performs 40 punctures with one stroke.

The multiple puncture BCG vaccination produces rarely local necrosis or permanent blemish.

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Komplikationen durch die Behandlung mit Sulfanilamidverbindungen, Verwicklungen vonseiten des peripheren Nervensystems.

Von

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Die zunehmenden Erfahrungen mit der Wirkung der Sulfanilamidverbindungen haben einerseits diesen neuen Chemotherapeutika einen Ehrenplatz unter den früheren Medikamenten verschafft, andererseits haben sie aber auch erkennen lassen, dass ihre Wirkung nicht immer unschuldig ist.

Wir hatten Gelegenheit, einige schwere Komplikationen der modernen Chemotherapie näher zu studieren, insbesondere solche, die sich auf die Funktionen des peripheren Nervensystems beziehen, und es erscheint uns angebracht, über diese Befunde im Zusammenhang mit den in der Literatur beschriebenen zu berichten.

Nach der Einführung der Sulfanilamidverbindungen in die Therapie stellte sich alsbald heraus, dass nach Verabreichung von Uliron periphere Neuritiden auftreten können. Diese klinischen Beobachtungen wurden durch die Ergebnisse experimenteller Untersuchungen bestätigt. So konnten Bieter u. M. periphere Nervenveränderungen bei Kücken feststellen, die einige Wochen lang Uliron enteral verabreicht erhielten (0.5—1 g pro kg Körpergewicht täglich). Pathologisch-anatomisch zeigten sowohl die Achsenzyylinder als das Myelin der peripheren Nerven ausgesprochen krankhafte Veränderungen, während überdies Demyelinisation und

Zellerfall im Rückenmark festgestellt wurden. Ferner konnten Hüllstrung und Krause mit Neo-Uliron (D. B. 87) Polyneuritis bei Tauben hervorrufen.

Inzwischen hatte man gefunden, dass auch durch Ultraseptyl (Sulfamethylthiazol) neurologische Ausfallserscheinungen entstehen konnten. So beschrieb in Holland Klessens eine Beobachtung, wobei nach intraglutealen Einspritzungen von Ultraseptyl Lähmungen im Gebiet des Plexus lumbosacralis auftraten, während von amerikanischer Seite darauf hingewiesen wurde, dass nach Verabreichung von Ultraseptyl in 4 % der Fälle Neuritiden vorkommen sollen. In seiner Auseinandersetzung über die neue Chemotherapie äussert Lindeboom die Annahme, dass die CH_3 -Gruppe, die in Uliron und Ultraseptyl vorhanden ist, vielleicht für die Entstehung dieser Neuritiden verantwortlich gemacht werden muss.

Bei den Versuchen von Bieter stellte sich aber heraus, dass auch das Sulfathiazol, worin keine CH_3 -Gruppe vorkommt, imstande ist bei Kücken Erscheinungen von Polyneuritis hervorzurufen. Auch die Beobachtung von Evans, die von Bieter mitgeteilt wird, beweist, dass Sulfathiazol auch beim Menschen einen toxischen Einfluss auf das periphere Nervensystem ausüben kann.

Schliesslich verdienen die neurologischen Komplikationen, die nach Verabreichung von Sulfapyridin auftreten können, noch nähere Erwähnung.

Im Jahre 1939 berichteten Löffler, Hegglin und Maier über das Vorkommen einer Ischiadicusneuritis nach Behandlung mit Eubasin, während eine ähnliche Mitteilung von Plügge im Jahre 1940 erschien. In Holland berichtete Diepen über 3 Fälle von Ischiadicusparalyse, die nach intramuskulärer Verabreichung von Solu-Dagénan entstanden waren. Die Lähmung trat nur am injizierten Bein auf. Bei 2 der von ihm beschriebenen Fälle entwickelte sich die Ischiadicusneuritis nach einer einzigen Einspritzung, weshalb der Autor annimmt, dass ausschliesslich die intraglutäale Verabreichung des Dagénan für die genannte Erkrankung verantwortlich gemacht werden kann. Hierzu kann jedoch bemerkt werden, dass Bieter im Tierexperiment nachweisen konnte, dass nach enteraler Verabreichung von Sulfapyridin bei Kücken neurologische Erscheinungen allgemeinerer Art (nämlich eine leichte Polyneuritis) auftreten können.

Unsere eigenen Erfahrungen erstrecken sich auf 8 Patienten, von denen 4 schwere neurologische Symptome nach Verabreichung von Ultraseptyl, 3 nach Anwendung von Sulfapyridin und 1 nach Verabreichung von Sulfanilamid zeigten. Es folge nun die Wiedergabe der betreffenden Krankengeschichten:

Fall 1: Pat. A, ein junger Mann von 22 Jahren, litt von Jugend an an Schmerzanfällen im linken Oberbauch, die auf einer intermittierenden Hydronephrose beruhten. Bei der Untersuchung in den ersten Tagen des Februar 1942 wurde eine Sekundärinfektion der Harnwege festgestellt. Pat. erhielt die ersten 4 Tage $5 \times$ täglich 2 Tabletten, die folgenden 3 Tage 3×2 und hierauf 2 Tage 4×2 Tabletten Ultraseptyl. Wegen eines juckenden, rotfleckigen Exanthems wurde das Medikament abgesetzt, obwohl der Urin noch viel Eiterzellen und sporadisch Staphylokokken enthielt. Im ganzen waren 37 g Ultraseptyl in 9 Tagen eingenommen. Das Blut zeigte eine relative Lymphozytose von 44 %, andere toxische Erscheinungen wurden nicht beobachtet.

Eine Woche nach beendigter Ultraseptylkur bemerkte Pat., dass die Kraft in seinen beiden Füßen abnahm. Er konnte die Zehen weniger gut bewegen und auch nicht mehr auf den Zehen stehen. Einen Tag später war Pat. nicht mehr imstande die Füße zu heben. Er konnte auch nicht mehr gehen, weil seine Füße nach vorn fielen. Gleichzeitig wurden auch beide Hände schwächer, doch war die Veränderung an den Händen viel geringer als die an den Füßen. Schmerzen oder Paraesthesien wurden von ihm nicht wahrgenommen.

Status: Bei der neurologischen Untersuchung (März 1942) wurden folgende Abweichungen festgestellt. Beide Füße, die blau aussehen und sich kalt anfühlen, liegen in ausgesprochener Equinovarusstellung. Die Streckbewegungen des Fusses und der Zehen sind beiderseits völlig aufgehoben, während wohl noch eine geringe Beugung von Fuss und Zehen möglich ist. Das Heben des lateralen Fussrandes ist praktisch unmöglich. Die Bewegungen im Hüft- und Kniegelenk geschehen mit genügender Kraft, obwohl man den Eindruck bekommt, dass auch diese Kraft etwas gelitten hat. Die Gesäßmuskeln werden mit genügender Kraft angespannt. Die peripheren Nerven sind weder zug- noch druckempfindlich. Bei der objektiven Untersuchung werden geringe Sensibilitätsstörungen im Innervationsgebiet des N. peroneus superficialis festgestellt. Die Kniereflexe sind in normaler Stärke vorhanden, während beiderseits der Achillessehnenreflex und der Reflex von Strümpell fehlen. Ausserdem besteht eine auffallende Hypotonie.

Im Laufe der Beobachtung fällt auf, dass sich eine starke Atrophie der Unterschenkel- und Fussmuskeln entwickelt, und zwar am stärksten in der M. tibialis anticus- und peroneusgruppe.

Die elektrische Untersuchung ergibt beiderseits im Innervationsgebiet des N. peroneus communis und des N. tibialis Erscheinungen von totaler elektrischer Entartung.

Bei der Untersuchung der Arme werden leichte motorische Störungen im Innervationsgebiet des N. medianus gefunden. Allmählich entwickelt sich eine Atrophie des M. abductor pollicis brevis. Sensible Veränderungen werden an den Armen nicht festgestellt.

Zusammenfassend kann man also sagen, dass sich im Anschluss an eine Ultraseptylbehandlung eine schwere Polyneuritis entwickelt hat, wobei die motorischen Ausfallserscheinungen stark vorherrschen. Die Veränderungen sind an den Beinen am stärksten im Innervationsgebiet des N. peroneus communis ausgesprochen. Sie sind an den oberen Extremitäten gering.

Fall 2: Eine Beobachtung, die der soeben beschriebenen sehr ähnlich ist, konnten wir bei Pat. B machen, einem 38jährigen Mann, der am 9. Juli 1942 auf die innere Abteilung des St. Canisius-Krankenhauses aufgenommen wurde. Er war 14 Tage vorher an einer Pneumonie akut erkrankt, das Fieber war gefallen, jedoch kurz danach infolge einer komplizierenden Pleuritis wieder gestiegen. Pat. war schwerkrank, mit hohem Fieber und einem doppelseitigen sanguinolenten Exsudat, in dem segmentkernige Leukozyten und keine Bakterien gefunden wurden. Am 12. Juli wurden aus dem Blut haemolytische Streptokokken gezüchtet. Die Flüssigkeit in der linken Pleurahöhle verschwand; am 17. Juli wurden rechts 1200 cm³ blutige Flüssigkeit abgelassen, worin kulturell haemolytische Streptokokken nachgewiesen wurden. Vom 12. bis 17. Juli war Pat. mit Dagénan per os (32 g in 6 Tagen) und wegen bleibenden Fiebers vom 19. bis 29. Juli mit Prontosil per os (38 g in 11 Tagen) behandelt. Trotzdem hierauf die Temperatur lytisch sank, war am 29. Juli die Flüssigkeit in der rechten Pleurahöhle eitrig geworden (Kultur: Pneumokokken), es wurde deshalb eine Bülow-Drainage angelegt. Am 4. August waren die Blutkulturen negativ. Eine Woche nach Aufhebung der Bülow-Drainage bestand bei normaler Temperatur Eiterretention (bei der Probepunktion Eiter mit Pneumokokken), weshalb am 25. August eine Rippenresektion vorgenommen wurde. Am 2. September entstand unter hohem Temperaturanstieg eine Thrombophlebitis der linken V. femoralis. Aufs neue wurden Prontosiltabletten gegeben (14 g in 6 Tagen). Am 10. September waren die Blutkulturen negativ. Am 14. September entwickelte sich von der Empyemwunde aus ein Erysipel. Wiederholt traten Schüttelfröste auf. Unter Prontosilverabreichung per os vom 14. bis 24. September (45 g in 11 Tagen) fiel jedoch die Temperatur, um gleich darauf wieder zu steigen. Am 24. September wurde aus dem Blut ebenso wie aus dem Eiter eines subconjunctivalen kleinen Abszesses am rechten Auge der Staphylococcus aureus gezüchtet. Am 2. Oktober wurde mit Ultraseptyl per os begonnen (49 g in 9 Tagen) und, obwohl die täglich bestimmte Konzentration im Blute zwischen 9 und 11 mg % schwankte, wurde am 16. Oktober aus dem Blut wiederum der Staphylococcus aureus gezüchtet. Vom 18. bis 25. Oktober wurden intravenöse Trypaflavineinspritzungen gegeben (10—20 cm³ ½—2 %ig). Inzwischen war das Empyem geheilt und das Drain am 18. Oktober entfernt, das Fieber blieb aber intermittierend mit

Schüttelfrösten bestehen. Gegen die allgemeine Staphylokokkensepsis wurde vom 26. Oktober bis 2. November wiederum Ultraseptyl verabreicht, was meistens per Injektion geschehen musste, weil die Tabletten ausgebrochen wurden. In 8 Tagen wurden 29 g gegeben, davon 12 g per os und 17 g intramuskulär. Innerhalb 2 Tagen verschwand das Fieber, der Ultraseptylgehalt des Blutes wechselte von 8—10 mg %. Am 6. November blieben die Blutkulturen steril, die Sepsis war überwunden, und es wurde die langdauernde Rekonvaleszenz des schwerkranken Mannes eingeleitet.

Obwohl alle Chemotherapeutika vorher ohne irgendwelche Störung (ausser Übelkeit) vertragen waren, entwickelte sich 15 Tage nach Beginn der letzten Ultraseptylbehandlung eine schwere Polyneuritis an den Beinen, die mit leichten Polyneuritiserscheinungen an den Armen vergesellschaftet war.

Bei der objektiven neurologischen Untersuchung zeigte sich, dass auch hier eine symmetrische Lähmung im Gebiet des N. peroneus communis und des N. tibialis bestand.

Im Gegensatz zu der erstbeschriebenen Beobachtung waren in diesem Falle sowohl die motorischen, als auch die sensiblen und trophischen Störungen stark ausgesprochen. Der N. ischiadicus war stark zug- und druckempfindlich, während an den Unterschenkeln und Füßen deutliche (vitale und gnostische) Gefühlsstörungen im Gebiet des N. cutaneus surae lateralis und medialis, des N. peroneus superficialis und des N. suralis vorhanden waren. Die Unterschenkel- und Fussmuskeln zeigten Erscheinungen von totaler elektrischer Entartung. An den Armen wurden leichte motorische Störungen im Innervationsgebiet des N. ulnaris und medianus festgestellt. Subjektiv klagte Pat. über Paraesthesien in den Fingern. Sensible Ausfallserscheinungen fehlten.

Vergleichen wir diese Beobachtung mit der vorhergehenden, so waren in diesem Falle die motorischen, sensiblen und trophischen Störungen der Polyneuritis gleichmässig stark zur Entwicklung gekommen.

Fall 3: Pat. C, 21 Jahre alt, wurde am 27. Oktober 1941 mit folgender Anamnese ins St. Canisius-Krankenhaus aufgenommen: Er war früher stets gesund gewesen, bekam aber Anfang September 1941 eine Eiterung am linken kleinen Finger. Mitte September entstand ein Furunkel im Nacken, hierauf noch 2 andere und Mitte Oktober noch einmal 3. Er fühlte sich dabei nicht krank, ein paar Tage nach der Heilung stellten sich aber ungefähr am 20. Oktober Schmerzen in der rechten Hüfte ein, die auf verschiedene grosse Gelenke übergriffen. Seit dem 20. Oktober war er bettlägerig, hatte hohes Fieber und phantasierte. Die grossen Gelenke waren schmerzhaft; eine Probepunktion der geschwollenen rechten Hüfte ergab keinen Eiter. Es bestand offenbar eine schwere Sepsis unter kontinuierlichem Fieber von 39°; am 3. November wurde aus dem Blut auf allen Nährböden Staphylococcus aureus gezüchtet. Am 4. November wurde mit der Ultraseptylbehandlung begonnen (6 mal täglich 2 Tabletten). Nach 4 Tagen fiel die Temperatur, stieg aber bei gleichbleibender Medikation 2 Tage später wieder, und am 12. November trat ein Schüttelfrost auf.

Die Behandlung wurde abgebrochen, nachdem in 8 Tagen 48 g Ultraseptyl per os verabreicht waren, und hierauf während 5 Tagen ohne irgendwelchen Erfolg Trypaflavin intravenös eingespritzt. Trotz Prontosilbehandlung vom 17. bis 25. November (31 g in 9 Tagen), intravenösen Rivanoleinspritzungen vom 26. bis 30. November und einigen Bluttransfusionen traten fortgesetzt Schüttelfröste auf. Das Fieber blieb intermittierend, der Allgemeinzustand wurde schlechter, ohne dass sich irgendwo im Körper ein lokaler Prozess zeigte. Am 16. und am 30. November wurde aus dem Blute wiederum auf allen Nährböden *Staphylococcus aureus* gezüchtet. Vom 1. Dezember ab bekam Pat., der alle Medikamente ohne die geringste Intoxikationserscheinung vertragen hatte, täglich grosse Dosen Ultraseptyl, nämlich alle 4 Stunden 3 Tabletten oder 1 ½ g, bei Erbrechen wurde 1 g intramuskulär und ½ g per os gegeben. Innerhalb 2 Tagen verschwand das Fieber, nach 3 Tagen wurde die Dosis herabgesetzt und in 9 Tagen im ganzen 52 g Ultraseptyl verabreicht, davon 34 g per os und 18 g intramuskulär. Die Temperatur blieb normal. Am 8. Dezember ergaben die Blutkulturen kein Wachstum. Obwohl der Allgemeinzustand viel besser geworden war, kamen am 20. Dezember auf den Blutkulturen wieder einige Kolonien von *Staphylococcus aureus* zum Vorschein, und als in den folgenden Tagen die Temperatur auf 38° stieg, wurde zur Verhinderung eines Rezidivs mit einer leichteren Ultraseptylkur begonnen. Unter 6mal täglich 2 Tabletten wurde die Temperatur sofort wieder normal und erfolgte, nachdem in 11 Tagen im ganzen 47 g per os gegeben waren, vollständige Wiederherstellung von der schweren Septikämie. Am 5. Januar 1942 zeigten die Blutkulturen kein Wachstum. Unter dem Einfluss der Sepsis hatte sich während des Krankheitsverlaufs eine sekundäre Anämie entwickelt, die inzwischen zurückgegangen war, ungünstige Nebenwirkungen des Medikaments auf das regelmässig kontrollierte Blutbild waren jedoch nicht gefunden worden.

Im Anschluss an die letzte Ultraseptylbehandlung begann Pat. über Schmerzen im Verlauf des rechten N. ischiadicus zu klagen, auch bestand links eine leichte Reizung im sensiblen Innervationsgebiet des genannten Nerven. Weiterhin entwickelte sich ein Schwächezustand in den Unterschenkeln und Füßen, der rechts mehr ausgesprochen war als links.

Bei der objektiven Untersuchung wurde beiderseits eine schlaffe Lähmung der Streckmuskeln des Unterschenkels festgestellt, ausserdem eine Lähmung des M. peroneus longus und brevis. Rechts ist die Lähmung stärker als links. Die Wadenmuskeln zeigen keine Abweichung. Die Knie- und Achillessehnenreflexe sind links und rechts gleich. Es besteht eine Atrophie in der Tibialis anticus- und Peroneusgruppe, rechts stärker als links. Bei der Sensibilitätsuntersuchung ist der rechte N. ischiadicus zugempfindlich, während ausserdem eine leichte Hypaesthesie im Gebiet des N. peroneus superficialis auffällt. Daneben bestehen trophische Störungen an den Füßen (kalte, blaue Füße, die schilfern). Bei der elektrischen Untersuchung zeigen die gelähmten Muskeln des rechten Beins Erscheinungen von partieller elektrischer Entartung. Die Liquor- und serologische Untersuchung ergeben keine Besonderheiten.

Epikrise: Pat. zeigt das Bild einer doppelseitigen Neuritis des N. peroneus communis, die im Anschluss an eine Ultraseptylbehandlung entstanden ist. Im Vordergrund stehen diesmal die motorischen Ausfallserscheinungen. Die neurologischen Symptome sind 22 Tage nach Beginn der Ultraseptylbehandlung aufgetreten.

Fall 4: Der 19jährige junge Mann D hatte am 10. April 1942 einen Furunkel am Halse bemerkt und war am 14. April mit Fieber und einer schmerzhaften Anschwellung rechts und links im Nacken erkrankt. Am 16. April wurde er in schwerkrankem, septischem Zustand auf die innere Abteilung des St. Canisius-Krankenhauses aufgenommen. Bei Inzision entleerte sich dicker Eiter, in dem Staphylokokken gefunden wurden. Aus dem Blut wurde auf allen Nährböden derselbe Mikroorganismus gezüchtet. Sofort nach der Aufnahme wurde mit einer kräftigen Ultraseptylkur begonnen, einen Tag lang alle 4 Stunden 3, danach alle 4 Stunden 2 Tabletten und später weniger; im ganzen wurden in 7 Tagen 44 g per os gegeben. Obwohl der Ultraseptylgehalt des Blutes zwischen 9.5 und 7 mg % schwankte, blieb das Fieber dauernd über 39°. Auf eine anschliessende Trypaflavinkur fiel die Temperatur bis unter 38°; am 3. Mai waren die Blutkulturen negativ. Der Allgemeinzustand, der während des Temperaturabfalls etwas besser geworden war, verschlechterte sich wieder und wurde am 7. Mai sehr schlecht. Obwohl die Temperatur vom 5. Mai ab normal gewesen war, starb Pat. am 8. Mai. Die pathologisch-anatomische Diagnose lautete auf Septikopyämie mit zahlreichen kleinen Abszessen (bis höchstens Nussgrösse) in allen Lungenlappen, vor allem an der Aussenseite, dicht unter der Pleura.

Zwei Wochen nach Beginn der Ultraseptylbehandlung hatte sich ein Schwächezustand in beiden Oberarmen entwickelt, links etwas mehr ausgesprochen als rechts. Während Pat. seine Hände und Vorderarme gut bewegen konnte, war es ihm nicht möglich, seine Oberarme bis zur Horizontalen zu heben. Bei der objektiven Untersuchung hatte sich beiderseits eine Lähmung des N. axillaris herausgestellt. Die Armreflexe waren normal. Andere neurologische Veränderungen wurden nicht festgestellt.

Epikrise: Im Anschluss an eine Ultraseptylbehandlung, die 8 Tage dauerte und wobei Pat. im ganzen 44 g Ultraseptyl per os gebrauchte, entwickelte sich 2 Wochen nach Beginn dieser Behandlung eine doppelseitige Neuritis des N. axillaris. Das Auftreten dieser Neuritis im Anschluss an eine Ultraseptylkur macht es wahrscheinlich, dass nicht die Sepsis, sondern vielmehr die toxische Wirkung des Ultraseptyl dafür verantwortlich gemacht werden muss.

Fall 5: Pat. E., 53 Jahre alt, wurde am 5. Juli 1942 auf die innere Abteilung mit Erscheinungen einer kruppösen Pneumonie aufgenommen.

Es wurde sofort mit der Dagénanbehandlung begonnen. Während der ersten 2 Tage bekam er 12 Ampullen Solu-Dagénan und nach dem Abfallen des Fiebers noch ein paar Tage lang 4mal 2 Tabletten Dagénan. Im ganzen wurden 12 g Solu-Dagénan intraglutäal und 15 g Dagénan in 6 Tagen per os gegeben.

Ungefähr 2 ½ Wochen nach Beginn dieser Sulfapyridinbehandlung trat ein schmerzhaftes Gefühl in der linken Gesässgegend auf, das mit einer Bewegungsstörung einherging.

Bei der objektiven Untersuchung wurde eine schlaffe Lähmung der linken Gesässmuskeln (Parese des N. glutaesus superior und inferior) festgestellt, während auch die Kraft der Streckmuskeln des linken Oberschenkels (Parese des N. tibialis) und der Wadenmuskeln abgenommen hatte.

Bei der Sensibilitätsuntersuchung war das Lasègue'sche Symptom stark positiv, während ausserdem eine erhöhte Druckempfindlichkeit des linken N. ischiadicus in der Gesässfalte und in der Kniehöhle auffiel. Der linke Achillessehnenreflex fehlte. Die paretischen Muskeln zeigten partielle elektrische Entartungsreaktion.

Epikrise: Im Anschluss an eine Sulfapyridinbehandlung (teilweise intraglutäal, teilweise oral) entwickelte sich eine Neuritis des N. glutaesus superior und inferior, ausserdem eine Neuritis ischiadica mit Freibleiben des N. peronaeus communis.

Fall 6: Pat. W, 44 Jahre alt, wurde am 27. Dezember 1941 komatös in die neurologisch-psychiatrische Klinik mit einem Status epilepticus aufgenommen.

Nachdem der Status epilepticus gewichen war, zeigte die weitere Untersuchung, dass Pat. an einer Hypertonie (240/140 mm Hg nach Riva-Rocci) mit leichter Herzvergrösserung nach links litt. Im Urin und im Harnsediment wurden nach Ablauf des Status epilepticus keine Abweichungen gefunden. Der Harnstoffgehalt des Blutes betrug 560 mg pro Liter (Ambard). Der Augenhintergrund zeigte verengerte Netzhautarterien. Da keine Anknüpfungspunkte für die Annahme einer Nephritis vorhanden waren, wurde die Diagnose auf essentielle Hypertonie, zerebrale Gefässkrämpfe und Pseudouraemie gestellt. Einige Tage nach der Aufnahme wurden geringe Abweichungen der Lunge festgestellt. Um die Entstehung einer Pneumonie zu verhindern, bekam Pat. während 3 Tagen Solu-Dagénan intraglutäal (12 g in 3 Tagen).

7 Tage nach Beginn der Sulfapyridinbehandlung entwickelte sich eine Peronaeuslähmung links, neben einer Lähmung des N. tibialis. Ausserdem fielen Sensibilitätsstörungen im Gebiet des N. peronaeus superficialis auf. Die Liquor- und serologische Untersuchung ergaben nichts Besonderes.

Während der weiteren Beobachtung entwickelte sich eine ausgesprochene Atrophie aller Muskeln des linken Unterschenkels und der kleinen Muskeln des linken Fusses. Die gelähmten Muskelgruppen zeigten eine totale elektrische Entartungsreaktion.

Epikrise: Im Anschluss an eine intraglutäale Solu-Dagénan-Behandlung entwickelte sich 7 Tage nach Beginn derselben eine schwere Neuritis des N. peronaeus communis und des N. tibialis. Neben motorischen Störungen bestanden geringe sensible Abweichungen.

Fall 7: Pat. G., 54 Jahre alt, wurde am 29. Oktober 1942 auf die innere Abteilung des St. Canisius-Krankenhauses mit akuter, fieberhafter Enterocolitis aufgenommen. Am 7. November entwickelte sich eine Thrombophlebitis am linken Bein. Nach einer Lungenembolie, die am 9. Dezember auftrat, entstand unter hohem Fieber ein grosser Infarkt im linken Unterlappen. Pat. war schwerkrank und bekam vom 11. Dezember ab Dagénan, erst 3mal und nach dem Abfallen der Temperatur 2mal täglich 2 Tabletten, im ganzen 12 g in 5 Tagen.

18 Tage nach Beginn der Dagénanbehandlung bekam Pat. heftige Schmerzen im Gebiet des linken N. ischiadicus. Auch im rechten Bein fühlte sie ischiasartige Schmerzen, diese waren aber weniger ausgesprochen als links. Gleichzeitig mit den Schmerzen bemerkte Pat., dass sie ihren linken Fuss nicht mehr bewegen konnte. Einige Tage später entstanden Schmerzen im Gebiet des linken Plexus brachialis.

Bei der objektiven Untersuchung war das Lasègue'sche Symptom beiderseits positiv (links stärker als rechts). Die Muskeln des linken Unterschenkels waren gelähmt, während rechts die Kraft, mit der der Fuss gestreckt und gebeugt werden konnte, stark herabgesetzt war. Auf beiden Seiten war der Achillessehnenreflex nicht auszulösen. Die Untersuchung der Sensibilität ergab eine doppelseitige Hypaesthesia im Gebiet des N. peronaeus superficialis. Schliesslich sei noch erwähnt, dass an den Füssen und Unterschenkeln trophische Störungen auffielen. Andere neurologische Ausfallserscheinungen wurden nicht gefunden, bis auf eine Zug- und Druckempfindlichkeit des linken Plexus brachialis.

Fasst man die genannten Erscheinungen zusammen, so muss man feststellen, dass sich bei dieser Patientin im Anschluss an eine orale Dagénanbehandlung (12 g in 5 Tagen) eine Polyneuritis entwickelt hat.

Da seit Beginn der Erkrankung noch zu kurze Zeit verstrichen ist, haben wir uns über den Verlauf dieser Polyneuritis noch kein Urteil bilden können.

Fall 8: Pat. H., 41 Jahre alt, wurde am 9. September 1942 wegen multipler Sklerose, die bereits 8 Jahre bestand, in die neurologisch-psychiatrische Klinik aufgenommen. Sie zeigte den klassischen Charcot'schen Symptomenkomplex, nämlich

- 1) spastische Paraparese der Beine,
- 2) Nystagmus,
- 3) skandierende Sprache,
- 4) Intentionstremor.

Während ihres Aufenthaltes in der Klinik entwickelte sich eine Cystitis. Aus dem steril aufgefangenen Urin wurden wiederholt Colibazillen gezücht-

tet. Pat. wurde deshalb mit Sulfanilamid behandelt, und zwar bekam sie 5 Tage lang täglich 6mal 0.3 g oral. 17 Tage nach Beginn der Sulfanilamidbehandlung entwickelte sich eine linkssseitige Neuritis des N. tibialis und des N. peroneus.

Die Streckmuskeln des linken Unterschenkels, die Peroneasmuskulatur und die Wadenmuskeln zeigten schon nach kurzer Zeit eine deutliche Atrophie, fibrilläre Zuckungen neben totaler elektrischer Entartungsreaktion.

Im Gebiet des N. peroneus superficialis bestand eine Hypaesthesia.

In den folgenden Monaten nahm die Atrophie stark zu.

Es lassen sich ungezwungen mehrere Argumente dafür anführen, dass diese Neuritis des N. peroneus communis und N. tibialis toxischen Ursprungs ist, und zwar:

1) Die Tatsache, dass Pat. kurz vorher mit Sulfanilamid behandelt wurde;

2) Das Auftreten der Neuritis 17 Tage nach Beginn der Behandlung. Aus den vorhergehenden Beobachtungen darf geschlossen werden, dass die toxische Neuritis gewöhnlich 2 bis 3 Wochen nach Beginn der Behandlung mit Sulfanilamidverbindungen auftritt;

3) Die klinische Übereinstimmung dieser Neuritis mit den vorherbeschriebenen. Nahezu in allen unseren Fällen bestand eine Prädisposition für den N. peroneus und N. tibialis;

4) Die Tatsache, dass das Bild einer peripheren Neuritis nicht in den Symptomenkomplex der multiplen Sklerose hineingehört;

5) Die Tatsache, dass man im Tierversuch mit Sulfanilamid Neuritiden hervorrufen konnte (Bieter).

Fassen wir nun die oben besprochenen Beobachtungen zusammen, so zeigt sich, dass nach Ultraseptylverabreichung (oral oder intramuskulär) in 3 Fällen eine ausgesprochene Polyneuritis an den Beinen auftrat, die mit oder ohne geringe Störungen an den oberen Extremitäten verlief.

Zweimal handelte es sich hauptsächlich um eine motorische Polyneuritis, während in einem Fall auch ausgesprochene sensible und trophische Störungen vorhanden waren. Im vierten Fall gab die Ultraseptylbehandlung den Anlass zur Entstehung einer doppelseitigen motorischen Axillarisneuritis.

2 Patienten bekamen im Anschluss an intraglutäale Verabreichung von Solu-Dagénan auf der Seite der Einspritzung eine Neuritis des N. ischiadicus. In einem Fall entwickelte sie sich nach oraler Verabreichung von Sulfapyridin (Dagénan). Bei einer Patientin trat nach oraler Sulfanilamidbehandlung eine einseitige, hauptsächlich motorische Neuritis des N. peroneus communis auf.

Legt man sich nun die Frage vor, wie man die Entstehung dieser Neuritiden erklären muss, so muss mit Bezug auf unsere Befunde unterschieden werden zwischen den Veränderungen, die im Anschluss an die Ultraseptyl- und orale Dagénanverabreichung entstanden, und denen, die nach einer parenteralen Solu-Dagénanbehandlung aufgetreten sind. Die Nervenentzündungen, die wir nach einer Ultraseptyltherapie sich entwickeln sahen, sind ohne Zusammenhang mit örtlichen Störungen, da das Ultraseptyl oral oder intramuskulär verabreicht wurde und die Erscheinungen auch ausserhalb des Einspritzungsgebietes auftraten. Man ist denn auch berechtigt, in diesen Fällen von einer toxischen Polyneuritis zu sprechen. Dasselbe gilt für die Polyneuritis nach oraler Dagénanverabreichung. Weniger sicher ist die Erklärung für unsere beiden Fälle von Ischiadicusneuritis nach Behandlung mit Solu-Dagénan. Doch sind unserer Meinung nach Argumente für die Erwägung von Diepen anzuführen, der es für nicht unwahrscheinlich hält, dass hier eine örtliche Einwirkung des Giftes auf den Nerv im Spiele ist (bei Solu-Dagénan). Hierfür spricht auch der Umstand, dass Klessens bei intraglutäaler Verabreichung von Ultraseptyl, wovon feststeht, dass es ein allgemeines Nervengift ist, dasselbe fand wie Diepen bei Solu-Dagénan. Zwar ergeben die Literaturdaten, dass das Sulfapyridin, oral oder intravenös verabreicht, beim Menschen wenig Schaden an den peripheren Nerven hervorruft, aus den Tierversuchen geht aber deutlich hervor, dass enterale Verabreichung von Sulfapyridin Polyneuritis zur Folge haben kann, womit seine toxische Wirkung auf das periphere Nervensystem im Tierversuch bewiesen ist. Hiermit stimmt unser Fall von Polyneuritis nach ausschliesslicher Verwendung von Dagénan per os vollkommen überein.

Wichtig ist nun die Frage, welche Prognose diese Gruppe von Nervenentzündungen hat. Auf verschiedenen Wegen kann man zur Beantwortung dieser Frage gelangen.

In erster Linie kann man sich durch die Resultate der elektrischen Untersuchung leiten lassen. Wie aus unseren Krankengeschichten hervorgeht, bestand bei Ultraseptylpolyneuritis in 2 Fällen, bei Solu-Dagénanneuritis in 1 Fall und ebenso bei Sulfanilamidneuritis eine totale elektrische Entartungsreaktion. Hieraus folgt, dass die Prognose in diesen Fällen ernst gestellt werden muss.

Dies stimmt auch vollkommen mit den Befunden überein, die bei der Nachuntersuchung nach längerer Zeit erhoben wurden.

Unser erster Patient zeigte nämlich im November 1942 noch stets eine nahezu vollständige Lähmung der Streckmuskeln und der Mm. peronei, während in den genannten Muskelgruppen noch eine totale elektrische Entartung festzustellen war. Die Behandlungsdauer unseres zweiten Patienten ist noch zu kurz, um über den klinischen Verlauf etwas sagen zu können.

Der dritte Patient, der Erscheinungen von partieller Entartung hatte, zeigte $\frac{3}{4}$ Jahr nach Beginn der Polyneuritis nur geringe Restsymptome, sodass hier eine günstige Prognose gestellt werden kann.

Über den Verlauf der doppelseitigen Axillarisneuritis haben wir uns kein Urteil bilden können, da der Patient kurz nach dem Auftreten derselben gestorben ist. Die Neuritis des N. ischiadicus nach Solu-Dagénan, die mit totaler elektrischer Entartungsreaktion einherging, ist nicht geheilt, Pat. zeigt zur Zeit (Dezember 1942) noch ein unverändertes Bild. Der zweite Fall von Neuritis ischiadica nach Solu-Dagénan mit partieller Entartung war 5 Monate später vollständig geheilt.

Um eine bessere Einsicht in die Art und den Verlauf dieser Nervenentzündungen zu erhalten, haben wir versucht die Ultra-septylpolyneuritis experimentell bei Tauben hervorzurufen.

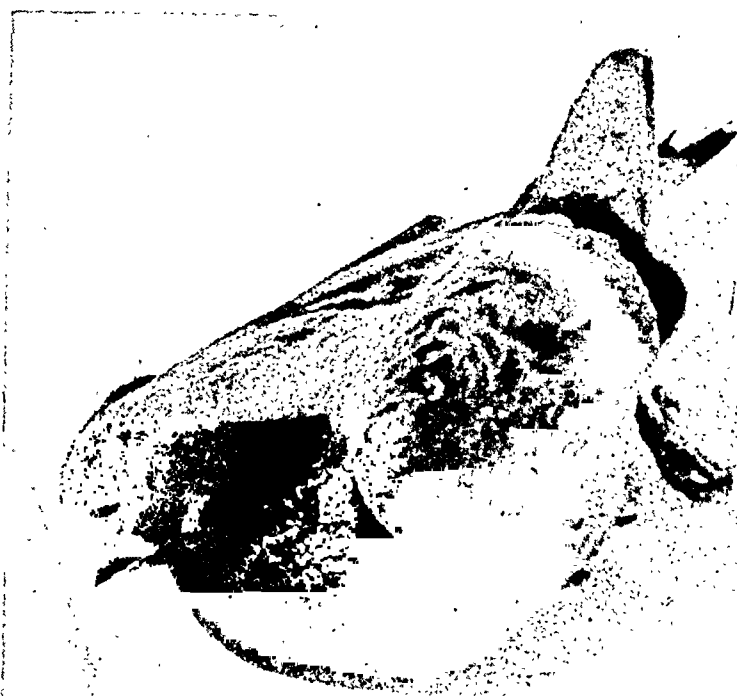
Bei einer Taube von 750 g wurde Ultraseptyl intramuskulär (in die Brustmuskeln) eingespritzt, Die Einzelheiten dieses Versuchs können folgendermassen zusammengefasst werden:

1. 4. 1942	intramuskulär	0.5 cm ³	=	100 mg	Ultraseptyl	
3. 4. »	»	0.5 »	=	100 »	»	»
5. 4. »	»	0.5 »	=	100 »	»	»
9. 4. »	»	1 »	=	200 »	»	»
11. 4. »	»	1 »	=	200 »	»	»
13. 4. »	»	1.5 »	=	300 »	»	»
15. 4. »	»	1 »	=	200 »	»	»
16. 4. »	»	1 »	=	200 »	»	»
17. 4. »	»	1 »	=	200 »	»	»
18. 4. »	»	1 »	=	200 »	»	»
20. 4. »	»	1 »	=	200 »	»	»

Am 20. 4. 1942 konnte das Tier nicht mehr fliegen, während seine Flügel schlaff vom Thorax abstehen. Bis zu diesem Tage sind im ganzen 2 g Ultraseptyl eingespritzt.



I. Beginnende Parese der Flügel.



II. Parese der Flügel und Füße.



III. Lähmung der Flügel nahezu komplett.



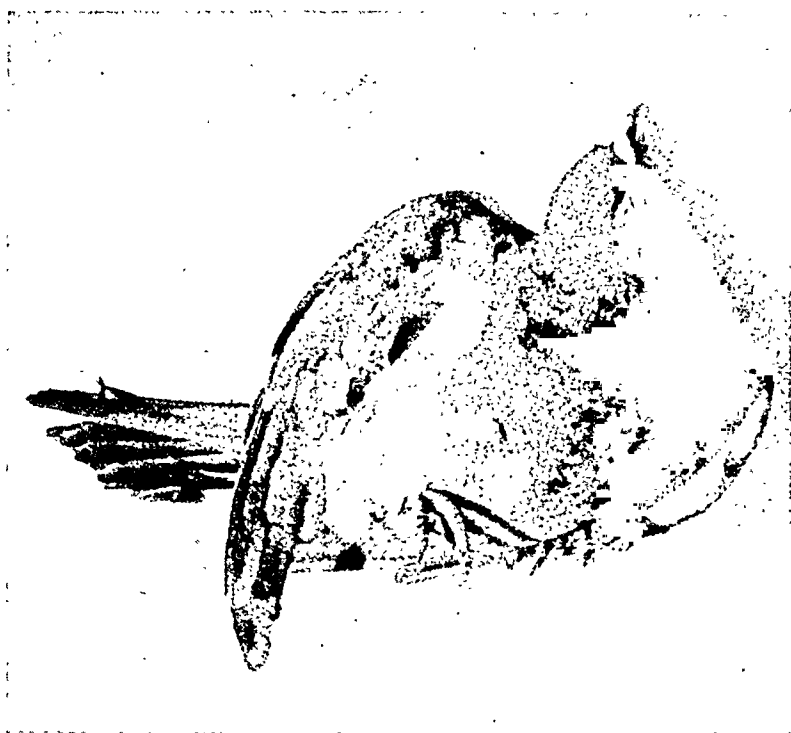
IV. Beginnende Parese der Halsmuskeln.



V. Zunehmende Lähmungserscheinungen der Rumpf- und Halsmuskulatur.



VI. Lähmung der Halsmuskeln stark ausgesprochen.



VII. Zustand kurz vor dem Tode.

Zwei Tage später (es sind dann im ganzen 2400 mg Ultraseptyl verabreicht) sinkt das Tier auf seinen Füßen zusammen und liegt manchmal stundenlang auf dem Bauch. Man bekommt den Eindruck, als ob Druck auf die Füße und die Flügel schmerzhaft ist. Zu gleicher Zeit fallen trophische Störungen auf.

In den folgenden Tagen nehmen die Lähmungserscheinungen zu. Die Kraft in den Füßen ist nun sehr gering, während die Flügel schlaff herabhängen. Das Tier liegt jetzt dauernd auf dem Bauch.

Anfang Mai 1942 (die Einspritzungen werden fortgesetzt) nimmt die Schwäche in den Flügeln zu. Am 27. Mai 1942 hängen beide Flügel gelähmt herab und sind auch die Füße vollständig gelähmt, während nun auch die Halsmuskeln paretisch sind (siehe Fotos).

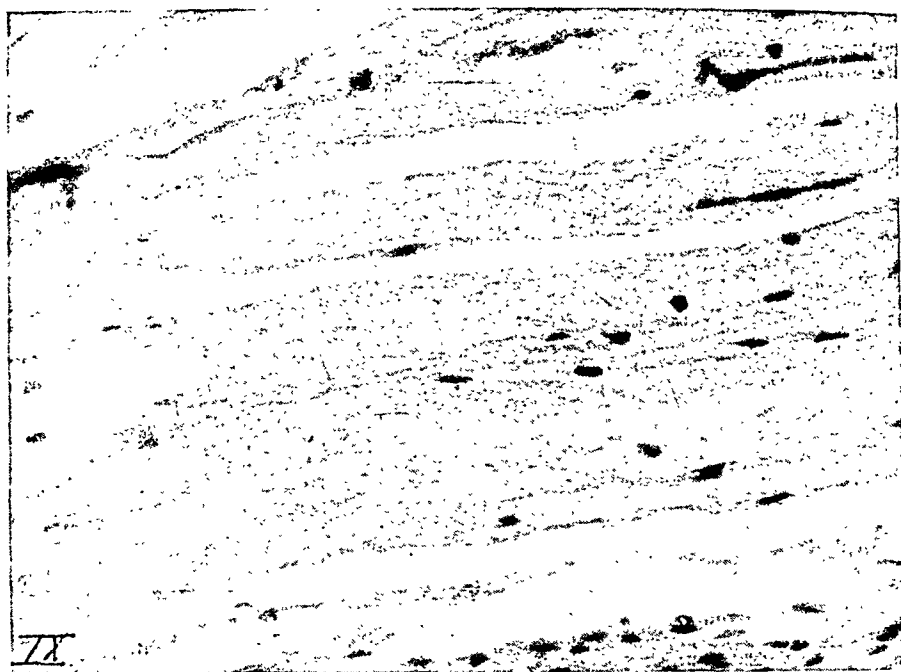
Während der letzten Versuchswochen wird der allgemeine Zustand des Tieres immer schlechter, und man bekommt den Eindruck, dass eine allgemeine Intoxikation besteht. Am 31. 5. 42 geht das Tier zugrunde.



VIII. Nervendegeneration. Sehr starke Schwellung und Vakuollisation der Myelinscheiden. N. ischiadicus.

Im ganzen wurden 10600 mg Ultraseptyl eingespritzt.

Fasst man den klinischen Verlauf bei diesem Versuchstier zusammen, so kommt man zu dem Schlusse, dass sich bei einer Taube nach intramuskulärer Verabreichung von im ganzen 2 g Ultraseptyl das Bild einer Polyneuritis entwickelt (schlaffe Paresen, druckempfindliche Nerven, trophische Störungen). Diese Polyneuritis nimmt bei Fortsetzung des Versuchs an Intensität zu. Die letzten Wochen bekommt man den Eindruck, dass das Tier, das anfänglich wenig krank ist, vollkommen vergiftet ist. Der Vollständigkeit halber betonen wir, dass nicht der geringste Grund bestand, eine Mangelpolyneuritis anzunehmen. Die pathologisch-anatomische



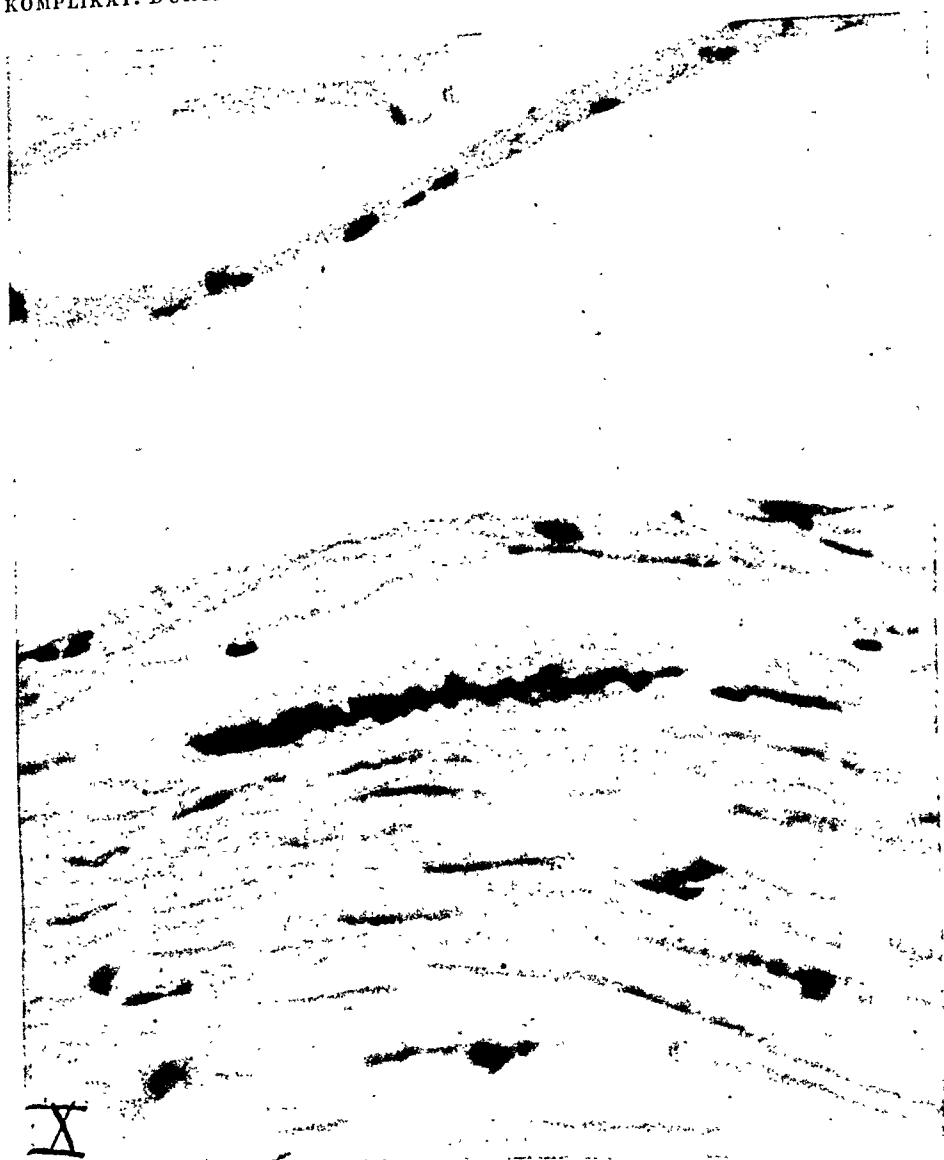
IX. Nervendegeneration. Markscheiden stark geschwollen und vakuolisiert. Einige Achsenzylinder zu deutlich sichtbar. N. ischiadicus.

Untersuchung (durch Dr. Mansens) bestätigte die klinische Diagnose. Die peripheren Nerven, besonders die der Füße, zeigten stark ausgesprochene krankhafte Veränderungen. Die Markscheiden waren stark geschwollen und deutlich vakuolisiert. An verschiedenen Stellen waren die Achsenzylinder stark geschwollen und zeigten einen abnorm geschlängelten Verlauf, stellenweise waren sie auch massig verklumpt. Die vier beigefügten Fotos geben ein deutliches Bild der beschriebenen pathologisch-anatomischen Verhältnisse.

Aus dieser pathologisch-anatomischen Beschreibung geht hervor, dass durch das Ultraseptyl ausgesprochen degenerative Veränderungen im peripheren Nervensystem hervorgerufen sind.

Überträgt man nun die Ergebnisse des Tierversuchs, sei es auch mit einiger Vorsicht, auf die menschliche Pathologie, so wird ohne weiteres deutlich, dass die Ultraseptylpolyneuritis eine sehr schwere sein kann.

Zu einer ähnlichen Schlussfolgerung kam auch Bieter, der bei Kücken eine Ultraseptylpolyneuritis erzeugen konnte. Bei



X. Nervendegeneration. Markscheiden stark geschwollen und gebläht. Achsenzylinder geschwollen und geschlängelt. N. ischiadicus.

einem Vergleich der experimentell hervorgerufenen Polyneuritis je nach Verabreichung von Sulfanilamid, Sulfapyridin, Sulfathiazol und Ultraseptyl zeigte sich, dass die pathologisch-anatomischen Veränderungen des peripheren Nervensystems am geringsten waren nach Verabreichung von Sulfanilamid und Sulfapyridin, stärker ausgesprochen nach Sulfathiazol, während die toxische Wirkung des Ultraseptyl auf das periphere Nervensystem besonders deutlich zum Ausdruck kam. Ausserdem stellte sich heraus, dass sowohl



XI. Nervendegeneration. Markscheiden stark geschwollen und aufgebläht. Achsenzylinder geschwollen, geschlängelt und stark verklumpt. N. ischiadicus.

Sulfathiazol, als auch Ultraseptyl neben Veränderungen im peripheren Nervensystem auch solche im Zentralnervensystem hervorrufen konnten.

Obwohl jeder Kliniker uns darin beistimmen wird, dass die moderne Chemotherapie unser therapeutisches Handeln auf ein höheres Niveau gebracht hat, liefern die mitgeteilten Befunde ungezwungen den Beweis, dass dieser wichtige Fortschritt auf therapeutischem Gebiet andererseits wiederum schwere und gefürchtete Komplikationen mit sich bringen kann.

In der Klinik haben wir den Eindruck erhalten, dass von allen Sulfanilamid-Präparaten das Ultraseptyl die grösste Gefahr bietet für das Entstehen einer Polyneuritis. Gsell geht bei seiner Beurteilung selbst soweit, dass er von der praktischen Anwendung des Ultraseptyls abraten zu müssen glaubt, und zwar auf Grund seiner Erfahrung in 151 Fällen, in denen er dieses Präparat angewandt hat. Er nennt es ein vorzüglich antibakteriell wirkendes Therapeutikum, das dieselben Eigenschaften hat wie das Sulfathiazol, aber nicht besser ist als dieses, auch nicht bei Staphylokokken-Infektionen; es hat dagegen als Nebenwirkung eine ausgesprochene Neigung zu Nervenschädigungen, meistens in der Form von Polyneuritis mit distalen Lähmungen. Die orale Resorption erfolgt nach seinen Untersuchungen langsamer als beim Sulfathiazol, die hohe Konzentration im Blute hält länger an und die Ausscheidung im Urin ist geringer mit Vorherrschen des acetylierten Anteils. Wegen der geringeren Urinkonzentration und der niedrigeren Ausscheidung des Sulfamethylthiazol infolge des stärkeren Abbaus im intermediären Stoffwechsel hält er es auch für weniger geeignet zur Behandlung von Infektionen der Harnwege als Sulfathiazol und Sulfapyridin.

Wir haben auch den Eindruck, dass das Ultraseptyl in bestimmten Fällen ausgezeichnete Resultate liefert, wir halten es aber für ratsam, gerade bei der Verwendung dieses Präparates mehr als bei der der anderen Chemotherapeutika mit dem möglichen Auftreten der hier beschriebenen Verwicklungen zu rechnen.

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Behaviour of the Nucleolar Apparatus during Growth and Differentiation of the normal Blood-cells in the Adult Stage.

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(Submitted for publication January 24, 1944).

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Introduction.

Among the cellular constituents of the blood, the erythrocytes are estimated to have a life of 30—60 days; the life of the granulocytes is stated to be 2—4 days only and that of the blood-platelets merely a few hours. In rats (6×10^6 red cells per mm^3) 3.10^6 erythrocytes are said to be delivered in one minute by the blood-forming organ into the blood stream. Thus even in an adult individual the bloodforming organ must have, in its specific cellular elements, the embryonal organ's intense capacity for growth.

Thus at every moment a considerable number of fully differentiated blood-cells of the same kind are produced from an original cell, having passed through a period of growth and, during that stage or subsequently, a differentiation. The various stages through which the bloodcell passes during the maturation process will be characterized by various degrees of growth and differentiation which are reflected in the cytological picture.

A summary account of the origin and development of the erythrocytes and granulocytes will be given below. In the normal adult mammal the blood formation is localized solely in the bone marrow, where the original tissue is to be regarded as an undifferentiated mesenchyme. The reticular cells are relatively small and have a faintly basophil cytoplasm, without any other specific substances observable in stained preparations. The nucleus is poor in chromatin. The first stage is marked by a transformation of the reticular cells into a stem-cell, during which the cytoplasm is being packed with basophil substances and mitochondria. The essential process in the maturation of the blood cells is the formation of hemoglobin or granula. Concurrently with the increase of the hemoglobin or granula, the cytoplasmic basophilia of the stem-cell diminishes. These processes are continuous; but, in accordance with the varying proportions of those substances as well as changes in the nuclear picture and cell volume, certain maturation stages in the development of the red and white cells have been defined and denominated.

The most animatedly discussed question in regard to the post-natal blood formation has been whether cells with an omnipotent reproductive capacity occur post-embryonally, or whether the stem-cells are already

endowed with a prospective potency in this respect. The differences of opinion on the subject have been on the whole bridged over by Doan, Sabin, and other investigators, with the following reasoning: — The mesenchyme, or the postfetal remnant thereof, produces special stem-cells for the blood formation. From these cellular units are developed certain cells, which function as primitive blood cells. According to the nature of the bloodforming tissue and of the resulting stimuli special stem-cells are developed; for example, in the bone marrow stem-cells for the granulocytogenesis (myeloblast) and in the lymphatic system lymphoblasts. According to this hypothesis, the originally mesenchymal forms of cell acquire in their specific tissue a capacity for differentiation.

The cytology of this further development from the stem-cell forms is well known. In this paper Pappenheim's nomenclature has been adopted for the granulocytogenesis: — 1. The myeloblast, also termed Pappenheim's lymphoidocyte, an undifferentiated, possibly polyvalent, stem-cell (Maximow's hemocytoblast.) 2. The pro-myelocyte, in which the first signs of the formation of granula appear (this group comprises also Pappenheim's leukoblast) 3. The myelocyte with distinct granula and nucleus matured to a pachychromatic chromatin structure, via 4 the. metamyelocyte, finally differentiated into 5. the definitive granulocyte.

In the erythropoiesis the corresponding distinctive stages are 1. the pro-erythroblast (Ellerman's erythrogonie) and 2. the erythroblast, a cell varying in type in regard to the appearance of the cytoplasm and the hemoglobin percentage, and which, after the disappearance of the nucleus, passes into the erythrocyte of the blood. (For further particulars the reader is referred to Maximow 1927).

As a large number of differentiated blood-cells are developed from a relatively small number of immature stem-cells, this course of development seems to afford an opportunity for studying, by suitable cytochemical methods, the endocellular processes of metabolism and any changes therein which may occur during the different phases of growth and differentiation. The maturation of the granulocytes seems to be particularly well suited for an investigation of this nature, as the degree of differentiation of the individual cell can be exactly determined by the continuous formation of granula and the characteristic transformation of the nucleus.

The protein-forming system in the cell.

The principal substances out of which a cell is built up, notably protein and nucleic acid, have characteristic absorption bands within the central range of the ultraviolet spectrum. This fact has been utilized by Caspersson for the localization and quantitative determination of these substances in individual cell structures. Facilities have thereby been afforded for studying the protein and nucleic-acid metabolism of the individual cell, especially during growth and division.

The results have shown, broadly speaking, that the self-reproducing system which is in operation in the individual cell during the formation of new protein substances (growth) consists essentially in the combination of protein with a polynucleotide group.

In the chromosome elements the accumulation of *ribodesoxynucleic-acid* during the pro-phase seems to effect the reproduction of gene protein. (Caspersson-Schultz 1938).

The occurrence of *ribose nucleotides* in the cytoplasm of rapidly growing cells, such as embryonal cells (Caspersson and Thorell 1941) and cancer cells (Caspersson and Santesson 1942) seems to be a phenomenon of universal validity (lit. in Caspersson 1941). In embryonal cells it was found that, with continued differentiation and diminished rapidity of growth, this high content of cytoplasmic nucleotides was gradually reduced.

The studies of Caspersson and Schultz have traced a connection between these two protein-forming systems. These authors have shown in *Drosophila* that the nucleic-acid metabolism of the cell is regulated by certain chromosome areas, namely the heterochromatin defined by Heitz and formerly regarded as inert. It was observed, for example, that the amount of cytoplasmic nucleotides in the ovum was affected by the amount of heterochromatin in the nucleus. When the nucleus contained an extra Y-chromosome, the amount of ribose nucleotides in the cytoplasm of the ovum showed an increase.

In the study of an extensive series of other material it was subsequently shown that certain chromatin sections which deviate in behaviour from the major part of the chromatin are associated with the protein-forming apparatus. These chromatin sections are characterized *chemically* by their large production of proteins

rich in hexone bases, during the interphase, in rapidly growing cells. Though, in view of the imperfection of the present cytological methods, it is not possible to determine how far these deviating chromatin sections actually correspond to Heitz' heterochromatin, that designation had been retained in the above-mentioned works (Caspersson and collaborators). Also in the material discussed below there seems to be but little prospect of cytologically determining the distribution of the Heitz' heterochromatin. In this paper therefore, the term »*nucleolus-associated chromatin*» (designating one of its most salient features) will be adopted instead (see below).

It should be made clear that we are not concerned here with the »*nucleolus-organising regions*» (even if those regions in certain material have been shown to lie in the above indicated parts of the nucleus). As was shown in regard to the heterochromatin in *Drosophila*, the part played by the nucleolus-associated chromatin in the formation of cytoplasmic protein seems to be, during the interphase, to produce ribose nucleotides and proteins rich in hexone bases, which gather into an acidophilically stainable, and ultraviolet-absorbing, nucleolus. In all types of cells in which large amounts of ribose nucleotides have collected in the cytoplasm, and in which protein-formation processes are going on, a well-developed nucleolus, consisting, *inter alia*, of proteins rich in hexone bases and varying amounts of ribose nucleotides, is found in the nucleus. Thus, for example, a large acidophil nucleolus is typical of the rapidly growing embryonal cell.

The reverse condition, i. e. cells in which the nucleolus is missing, as in male sex cells, is marked by the absence of a growing cytoplasm.

As a well-developed nucleolar system, i. e. a large nucleolus rich in hexone bases and ribose nucleotides as a rule surrounded by nucleolus-associated chromatin and large amounts of ribose nucleotides in the cytoplasm, is characteristic of cells in which a rapid synthesis of cytoplasmic proteins is taking place, the nucleolus-associated chromatin probably functions, *inter alia*, as a factor of primary importance for the formation of nucleotides and proteins in the cytoplasm.

The connection between this nucleolus system and the cytoplasmic nucleotide protein-forming system could directly be followed

on suitable material (ganglion cells from *Lophius piscatorius*), (Hydén 1943). Proteins rich in hexone bases were shown to migrate from the nucleolus towards the parts of the nuclear membrane where a formation of cytoplasmic ribose nucleotides is proceeding and, in connection therewith, a synthesis of cytoplasmic protein.

The formation of new protein is the central process in all cell growth. The mechanism «nucleolus-associated chromatin-nucleolus-cytoplasmic nucleotides» may therefore be regarded as a system of growth in the cell, serving for the formation of cytoplasmic protein. *Possibility is thus afforded for determining the rapidity of growth in a cell by examining the size and composition of the cell organelles functioning in the protein-forming system above referred to.* Special importance may be attached to the amount and composition of the nucleolar material as well as to the content in the cytoplasm of ribose nucleotides formed from the nuclear membrane, as these factors are most easy to judge. Thus a cell in rapid cytoplasmic growth may be roughly characterized as follows: A large nucleolus containing ribose nucleotides, a large nucleus with a well-developed nuclear membrane and a high concentration of ribose nucleotides in the cytoplasm. When the cell is developed by normal differentiation and ceases to form new cytoplasmic protein, the nucleolar apparatus is reduced, so that scarcely more than its original chromatin portion, observable as a chromocentre is left (Caspersson and Thorell 1941). The concentration of ribose nucleotides in the cytoplasm concurrently diminishes (for further particulars see l. c. and Caspersson 1941).

The first task in studying the development of the bloodcell is primarily to investigate whether the cytoplasmic protein-forming mechanism works in the same way as in the previously studied cells. Such investigation will be rendered possible by a comparative cytochemical investigation of the granulocytopoiesis and erythropoiesis (see below p. 357). One of the most marked disparities between corresponding cell generations in the myeloid and erythroid series is the great difference in the development of the cytoplasm. The former, after development from the stem-cell, acquire, in their earlier stages (the promyelocyte) a very large cytoplasmic mass, which during the differentiation, in connection with the in-

crease in the number of cells, is very gradually reduced (for further particulars see below). The cytoplasm of the erythroid cells, on the other hand, rapidly diminishes concomitantly with the differentiation. If, parallel with this distinction in the development of the cytoplasm, a difference in the development of the nucleolar-nuclearmembrane apparatus can be demonstrated, it may be concluded that the formation of cytoplasmic protein proceeds on similar lines as in the previously studied cells.

This is shown below to be the case. The possibilities thus afforded for judging, from the composition of the individual cell organelles, the rapidity of growth in the various stages of differentiation should also enable us to study the question whether the formation of blood cells in the adult bone marrow proceeds mainly by a differentiation starting from early stages or by homoplastic divisions of more differentiated types of cell. Certain questions bearing on the subject will be discussed below.

Some earlier observations regarding the cytology of hematopoiesis.

In the mechanism of cell growth above referred to, the central position is held by a special cell organelle, the nucleolus. Some earlier observations regarding its occurrence and development during the hematopoiesis will therefore be briefly summarized below.

The development of the nucleolar apparatus in certain blood-cell stages was studied by Butterfield in 1907. He found in material from myeloid leukemias that the non-granulated cells, supposed to be undifferentiated, all possessed nucleoli, though in varying numbers. The number had no connection with the size of the nucleus or of the cell itself: the only factor which seemed to be constant was the total mass of the nucleolar substance.

Between these undifferentiated cells and the typical granulated forms, he found all kinds of transitional stages, with continuously decreasing basophilia in the cytoplasm and an increasingly condensed chromatin network in the nucleus. In view of the density of this network, Butterfield considered that a nucleolus in the myelocyte could be discovered only with difficulty, but that it nevertheless existed.

Butterfield also investigated the reaction of these nucleoli to stains and observed in the nucleolar substance a marked affi-

nity for acid dyes. Under certain conditions, however, he found that they could be stained *also* by basic dyes.

Butterfield's observations induced him to endorse the view advanced by Heidenhain in 1907 that growing cells contain larger amounts of nucleolar substance than others. Thus, so far as concerned the typical kinds of cells occurring in myeloid leukemias, he regarded the number and size of the nucleoli as an indication of the state of proliferation of those cells.

Schridde (1907), in distinguishing between the granulocyte and lymphatic groups of stem-cells, describes the myeloblast contained in the normal bone marrow of man as a cell with a moderately large cytoplasm of spongy structure and equably distributed basophilia, having a round or oval nucleus with a distinct nuclear membrane. The chromatin structure, he states, is fine-meshed. Acidophil nucleoli, provided with a nucleolar membrane, often occurred in rather considerable numbers being distributed here and there in nucleus. He found this nucleolar membrane to be particularly marked in those nuclei which contained merely a single nucleolus. He also found that the myeloblast nucleoli corresponded well with Flemming's definition: round, sharply delimited formations, consisting of plastin and therefore differently stainable than the rest of the chromatin.

Schridde considered himself also to have found that all the nucleated cells in the bone marrow contained nucleoli. In addition to the above described findings of nucleoli, he was able with his special Altmann-Schridde staining method, to show the existence of nucleoli also in the differentiated cells. According to him, this method enabled nucleoli to be discerned even in advanced erythroblasts, though, with ordinary staining, they were obscured by a deeply stainable chromatin network.

Maximow and other authors, however, hold the most widely accepted view that later stages, with more or less condensed chromatin structure in the nucleus, are devoid of nucleoli.

According to Lambin (1923) the nucleolus is already missing in the myelocyte, whereas Maximow, for example, considers that this cell contains 2—4 irregularly formed nucleoli.

Pappenheim (1907, 1910) finds nothing characteristic in the number of nucleoli in the myeloblast. Klein (1910) considers that the myeloblast (the most undifferentiated hematopoietic cell) as

a rule has more nucleoli than the more differentiated cells, but nevertheless finds that the reverse is often the case. Downey actually states that cells with an otherwise typical myeloblast structure, but which are totally devoid of nucleoli, are occasionally observed.

Downey (1933) makes the following statement regarding the further fate of the nucleolus: »Nucleoli disappear during the early stages of differentiation of myeloblasts. In the red cell series this occurs very early, but in a series of developing granulocytes they may be carried over into the leucoblast and promyelocyte stages. In diagnostic work it is, therefore, very important to give special attention to the nucleoli, because their presence is an indication of immaturity of the cells, but they usually do not tell us whether the cell has originated in the marrow or lymphatic tissue.»

For other literature regarding the nuclear structure, the cytoplasmic basophilia and development of granula in the different cell stages, good summaries will be found in the cited works of Maximow, Downey, and others.

Some earlier cytochemical investigations of blood cells.

Grawitz and Grüneberg in 1906 investigated blood cells from man in the ultraviolet microscope in accordance with the method of Köhler. The wave-length adopted was Mg 2800 Å. They believed however that the absorption of the different cell structures was due to their mineral content. No conclusions, however, were drawn in regard to the chemical composition of the cell structures, this being due to the imperfect knowledge of those days regards the substances occurring in the cell and their absorption of light.

In the above-mentioned study of Caspersson and Thorell on the endocellular metabolism of protein and nucleic acid in embryonal tissue, certain stages in the development of the blood cell were analyzed with the method elaborated by Caspersson for taking absorption spectra of individual cell parts.

By comparisons with the thorough histological investigations made by Dantschakoff on the hematopoiesis of fowl embryos, the different bloodcell stages could easily be identified in the ultraviolet microscope. This procedure showed very distinctly that the primitive blood cells possessed a cytoplasm packed with intensely absorbing substance. The absorption measurements were made on

the cytoplasm of erythroblasts lying in the area vasculosa as well as on the cytoplasm of fully differentiated erythrocytes. The former, rapidly dividing, primitive forms had high concentrations of cytoplasmic nucleotides, whereas these were entirely lacking in the fully differentiated forms, which were not susceptible of further growth. Dantschakoff's statement that this blood-cell series is characterized by a diminishing tendency in the cytoplasm to take on basic dyes, was explained by the observation of Caspersson and Thorell that the amount of cytoplasmic nucleotides decreases during the differentiation.

II. Material.

The ultraviolet picture.

The nucleic acid group is characterized by a specially high absorption in ultraviolet light about 2600 Å, which is due to the pyrimidin rings of the bases contained in the nucleotides. As Caspersson (1936) has shown, this absorption band in cell material might be regarded as specific for the nucleotides (cf l. c.).

The distribution of these substances within a cell can thus be studied by measurements of absorption spectra of different cell parts. Due to the very high absorption of the nucleotides at 2600 Å even single micro-photographs taken under suitable conditions with light of this wave-length give some evidence. A condition is however that the cell itself does not contain structures which refract light so intensely as to distort the actual absorption within the cell. Such structures may either be normal substances, such as granula, or artefacts due to unsuitable fixing (see Caspersson 1936, 1940).

Other substances occurring in cells which show some, though fainter, absorption in ultraviolet light, and which consequently are brought out on an absorption micro-photograph, are proteins and certain sterols.

As Fig. 1 shows, the absorption of the nucleic-acid group at 2600 Å is considerably higher than that of proteins of ordinary contents of tyrosine, tryptophan and phenylalanine. Thus on a photograph taken at this wave-length the cell structures containing nucleic acid in comparative concentrations will stand out in sharp relief. A layer of a 10 per cent. protein solution 5 μ in thickness, with the usual composition of cellular protein, absorbs about

5 per cent. of the light falling in. This absorption corresponds to a 0.2 per cent. nucleotide solution. This shows that the dark areas on the photograph, corresponding to a high absorption of light in the cell cannot be due solely to the protein: They are in fact a direct indication of the presence of nucleic acid. It should be pointed out, however, that the presence of the nucleic-acid component

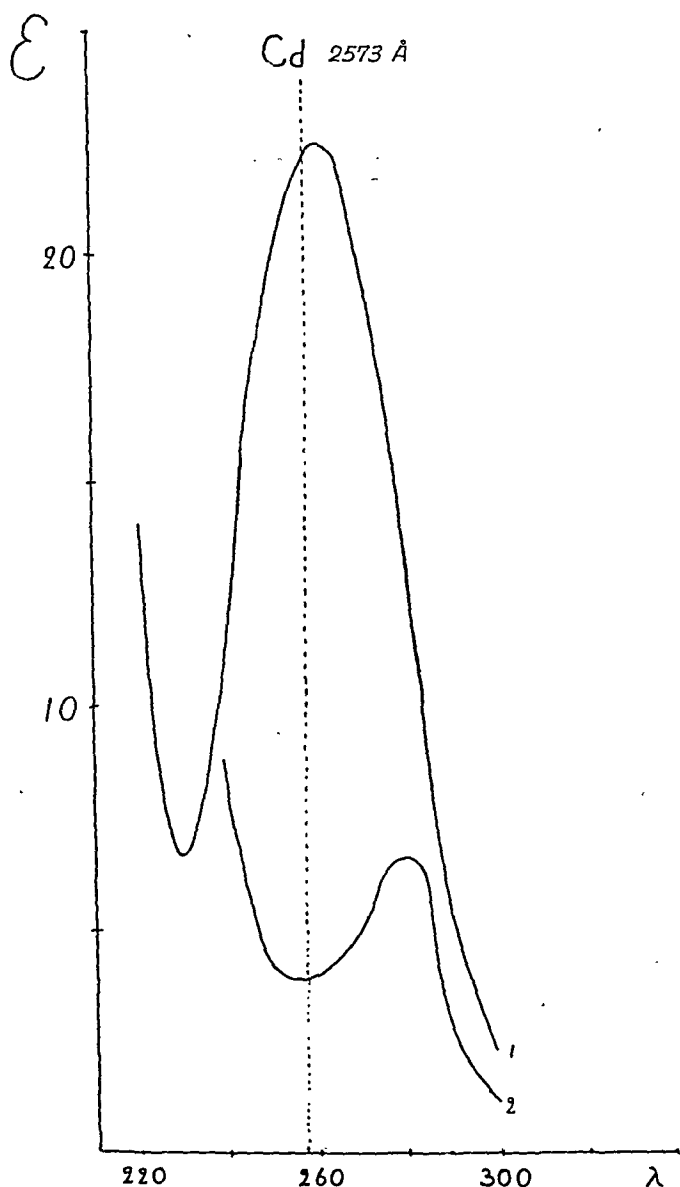


Fig. 1. Absorption curves of a 1 % solution of ribose nucleic acid (1) and a 10 % solution of serum-albumin (2). The figure shows the marked difference in absorption between the nucleic acid and the protein.

cannot be considered to have been proven when high absorption is found on the ultraviolet photograph, where indefinite factors, such as exposure and development, may come into play. *Only a detailed absorption spectrum of the cellular part in question can determine the matter.*

Certain of the sterol have their principal selective ultraviolet absorption at shorter wave-lengths (2300—2400 Å) and, as compared with the nucleotides containing pyrimidin bases, are scarcely noticeable on the ultraviolet photograph at 2600 Å.

Summing up, it may be stated that under certain conditions, and taking into account effects due simply to the refraction of light, the general endocellular distribution of nucleotides can be judged on an ultraviolet photograph, taken under proper condition as those areas which are rich in nucleotides, owing to their high absorption of light, show less density than the remaining areas. — A uniform plate material (Agfa Normal, see below) has been used throughout. Also the exposure, development and printing were kept as constant as was practicable without the adoption of special reference systems.

As the principal object of investigation, the author has used living cells from the femur bone marrow of rats. The left femur was dissected free from muscles and extracted, whereupon the proximal part was crushed so that the marrow was exposed. It was then dabbed on a wellcleaned quartz slide. A drop of Ringer's solution was added, a cover glass was put on, and, in order to prevent drying, the edges were smeared with vaseline. The preparation was examined as soon as possible in an ultraviolet microscope (Köhler 1931) with the use of the wave-length 2573 Å, thus very near the absorption maximum of the nucleic acids. For data regarding the microscope and photograph, see the explanatory text of fig. 2. The appearance of the different types of cells involved in the development of the blood cell is described below (for the nomenclature, see above).

The Myeloid Series (Fig. 2).

1. The Myeloblast.

A rather large, as a rule slightly oval cell. The cytoplasm, forming a fringe round the nucleus, has a somewhat irregular outline and contains a considerable amount of intensely absorbing substances. These substances as a rule are evenly distributed in the cytoplasm, though several small vacuoles are sometimes observed.

The nucleus is large, usually oval and bounded by a fine, but distinct nuclear membrane. The chromatin structure is relatively «light» and consists of an abundance of fine absorbing granules.

The nucleus moreover contains several (2—4) large, rather intensely absorbing nucleoli, which as a rule are evenly distributed and situated near the border.

2. The Promyelocyte.

A large, round cell. The large cytoplasm moderately absorbs the ultraviolet rays (for particulars see the quantitative determinations given below), but contains largish, more faintly absorbing areas, which in all probability are due to the formation of granula.

The nucleus is smaller than that of the preceding cell (see below), and provided with a fairly distinct membrane. The ultraviolet-absorbing chromatin is more or less accumulated in small clumps, often lying immediately within the nuclear membrane.

In addition, the nucleus contains 1—3 moderately large nucleoli, which intensely absorb ultraviolet light.

3. The Myelocyte.

A moderately large, round or oval cell. The cytoplasm has a granular structure and is relatively dark, presumably owing to the refractive effect of the abundant granula.

The nucleus is rather small, round or unevenly oval. No distinct nuclear membrane can be discerned. The chromatin is arranged in largish clumps.

As a rule a couple of distinctly absorbing largish formations can be observed in the nucleus.

4. The more or less mature granulocyte.

A small cell with the cytoplasm full of refractive granula, causing a considerable loss of light.

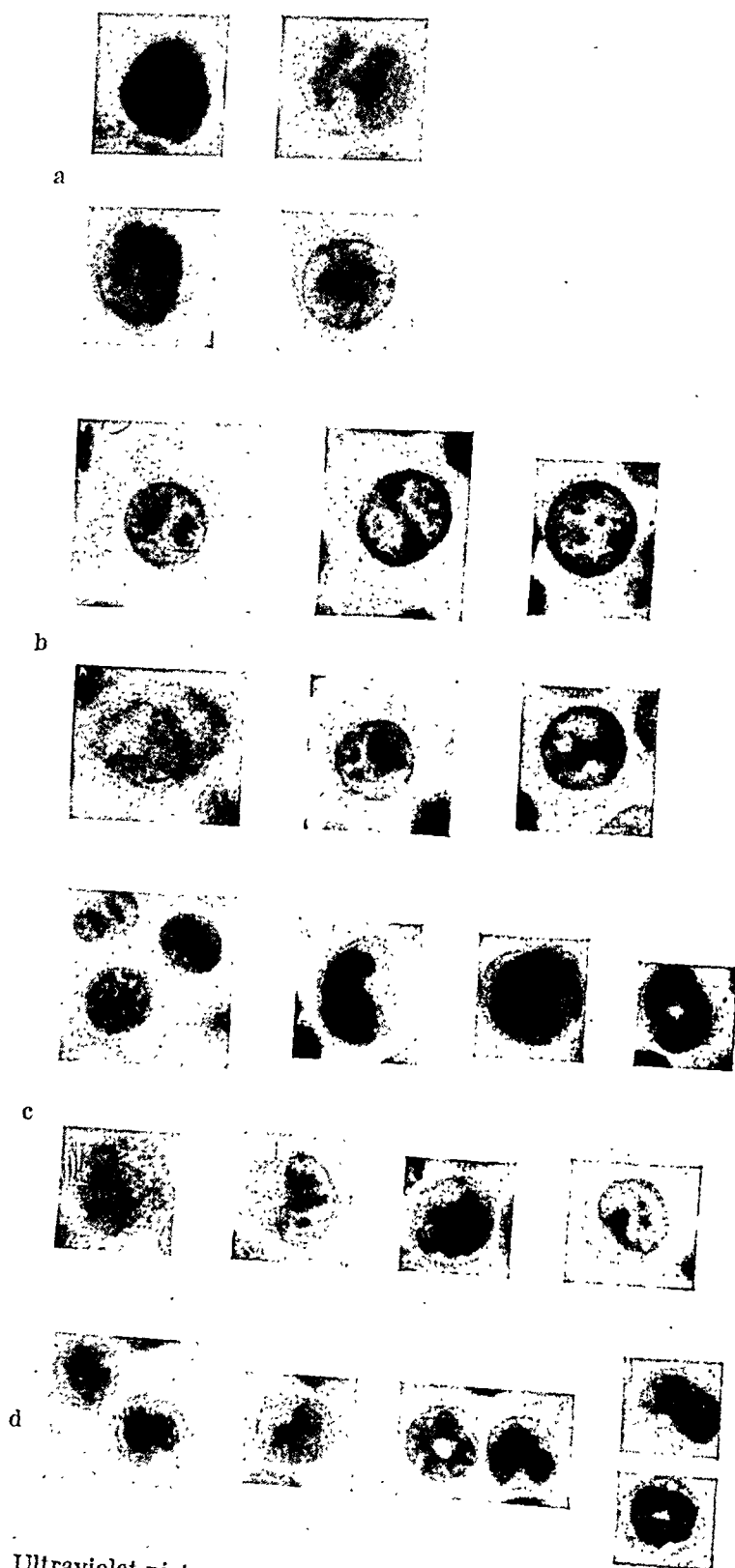


Fig. 2. Ultraviolet pictures of myeloid cells at different stages. Wave-length 2573 Å. Objective aperture 1.25. Condenser aperture 0.9. Magnification 1150 ×. Plate: Agfa Normal. Development: alkaline hydroquinon 5 minutes. The total ultraviolet material comprises 600 plates. Cells from 70 rats.

a) myeloblast b) promyelocyte c) myelocyte, d) granulocyte

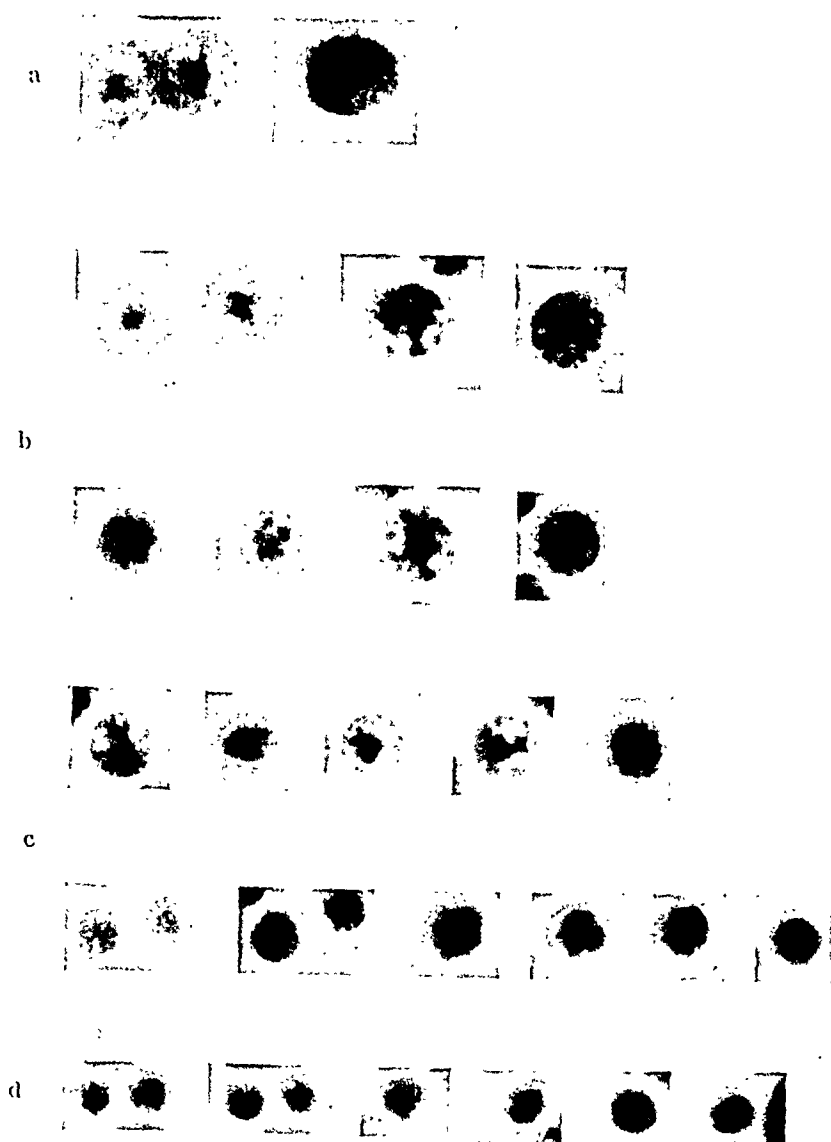


Fig. 3. Ultraviolet pictures of erythroid cells at different stages, Magnification 1150 \times . For other data see Fig. 2.
 a) erythroblast. b) basophilic erythroblast. c) polychromatic c. d) orthochr. c.

The nucleus first assumes an annular form (Di Guglielmo 1914) and subsequently, before the final lobed structure is developed, a rod shape. During this differentiation the absorbing chromatin gets increasingly caked. The nucleoli, or rather the chromocentre areas in these cells (see below), which down to the metamyelocyte can be clearly distinguished as small, intensely absorbing clumps, finally disappear altogether in the coarse chromatin structures.

The Erythropoietic Series (Fig. 3.)

1. The Proerythroblast.

A large, usually round, cell. The cytoplasm is rather scanty and throughout intensely absorbing.

The nucleus is round, with a thin, quite distinct nuclear membrane. The nuclear structure shows a picture of larger or smaller absorbing chromatin grains and, as a rule, two large, intensely absorbing nucleoli.

2. Erythroblasts of various stages.

The size of the cell diminishes rapidly with the maturation. Even at the stage which, to judge by the size and nuclear structure, follows immediately after the proerythroblast, the amount of absorbing substance in the cytoplasm has decreased considerably and during the following stages is maintained at a practically constant level, which is presumably due to the diminishing content of nucleotides and the increasing amount of hemoglobin.

The nucleus undergoes a pyknotic degeneration, manifesting itself in the concentration of absorbing substance. In the earlier stages the nucleoli are very imperfectly developed. In the more differentiated erythroid cells they are not developed at all. The rounded, intensely absorbing formations in the erythroblast nucleus must be regarded, according to previous investigations, as chromocentres. Towards the end of the differentiation process these formations are not visible at all, owing to the more or less coarsely packed chromatin (see below).

Quantitative determination of the cytoplasmic nucleotides.

A method for taking absorption spectra in individual cellular elements has been elaborated by Caspersson (see Caspersson 1940). By analysis of the absorption curves obtained, the content of nucleic acid and protein in these organelles can be estimated. But, owing to the small size of e. g. the intensely absorbing cytoplasm of the myeloblast, sufficient accuracy cannot be attained with this method.

The nucleotide concentration in the cytoplasm can, however, be approximately estimated by measuring the density of the cell

organelle on the ultraviolet photogram, as compared with the density of the surrounding free plate area. If one uses monochromatic light of the wave-length 2573 Å, in which the nucleic acids have their absorption maximum, the density, under otherwise constant conditions, will, practically speaking, depend entirely on the nucleotide concentration in the cell organelle (see above). The diminution of cytoplasmic nucleotides in the differentiation of the myeloblast into the promyelocyte should be determinable in this manner, seeing that in these cells the cytoplasm is homogeneous, whence the absorption is but little affected by the diffraction of light owing to interspersed refractive granula. In order to obtain comparable values, cytoplasmic areas with the same thickness of layer must be measured. This condition seems to be satisfactorily fulfilled by points near the nucleus.

The photograph was taken with a definite time of exposure (2 seconds). The density curve of the plate under the given conditions was known, whence the corresponding intensity could be deduced from the degree of density. At the same time the intensity value for a point lying close to the measuring area was determined, which value corresponded to the intensity of the light falling on the measuring area. The transparency of the measuring point is thus obtained from the ratio between the intensity value of the picture of the object and that of the environment. (For a discussion regarding the similarity of the picture to the object and the possibility of accurate measurement of the absorption of light in microscopic preparations, see Caspersson 1936).

In order to control the density curves of individual plates, a wedge with stepped, known, rising coefficients of extinction was photographed at the same time. By measuring the density for each wedge value and by tracing the density curve, a good control of the exposure and development of each plate was obtained.

The method of measurement was as follows: — The density measurement on the developed photograms (exposure, source of light and development as far as possible constant) was carried out as a measurement of transparency. The zero per cent. intensity value (minimum density) was computed by the transparency of unexposed plate area. The 100 per cent. intensity value (maximum density) was obtained by the measurement of areas completely free from substance. The relative value was computed by measurement

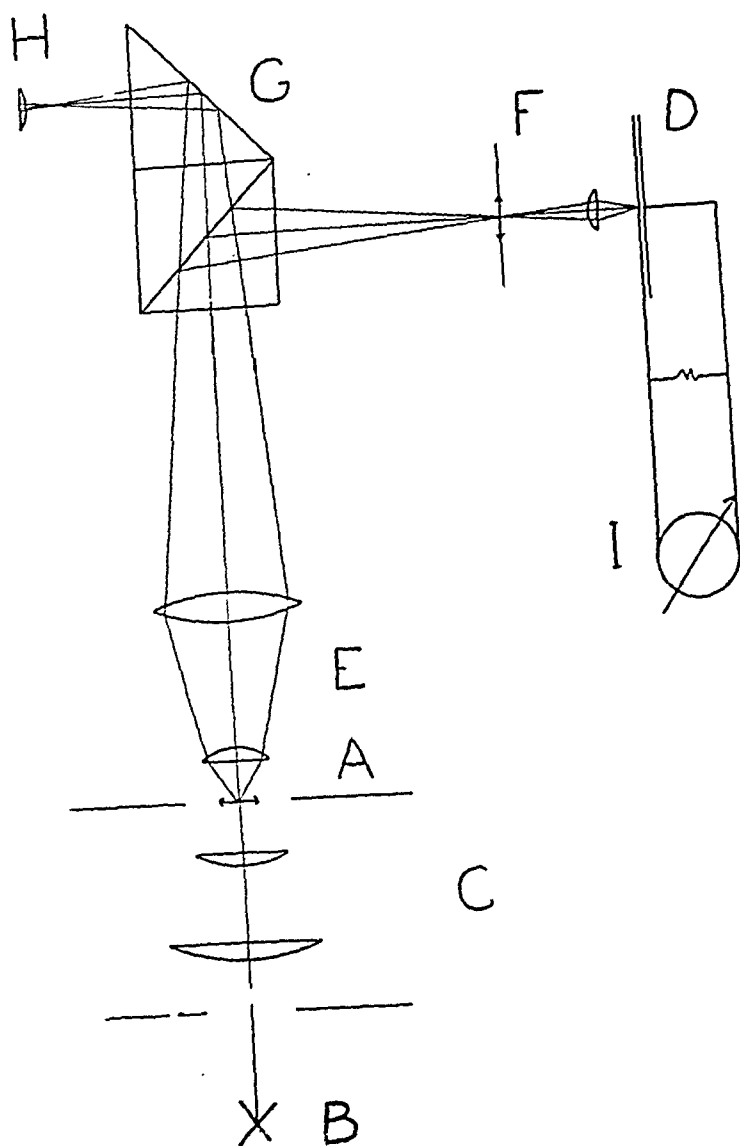


Fig. 4. Sketch of the micro-photometer used in measurements of density.
Explanation see text!

of a free area in the immediate vicinity of the measuring point of the cytoplasmic absorption.

The construction of the registering instrument was as follows: — The measured plate area (A) was illuminated by the source of light (B) through the condenser C. The light was cast at right angles to the plate. The influence of light scattered from surrounding plate areas was eliminated by a diaphragm which left only the measuring area free. The measuring area was projected on the selenium photo-

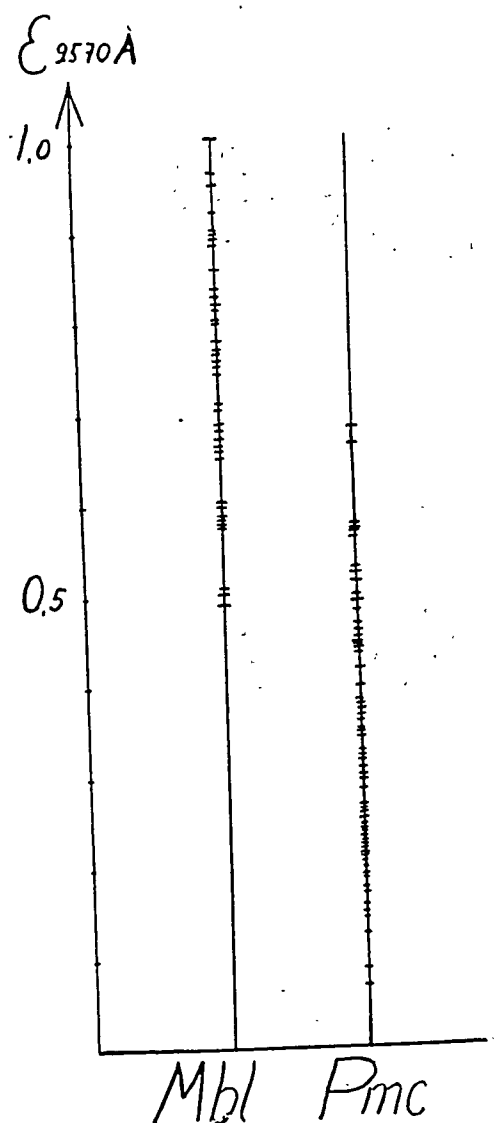


Fig. 5. The figure shows the striking difference in cytoplasmic absorption between the myeloblast and the promyelocyte at 2570 Å. This phenomenon can be explained only by a marked diminution of the cytoplasmic nucleotide concentration during development from myeloblast to promyelocyte. Every point indicates the extinction coefficient of the cytoplasm in a single cell. (Mbl = myeloblast, Pmc = promyelocyte).

cell D through the objective E and the diaphragm F. The prism G and ocular H is a centring arrangement. The photo-current arising from the illumination of the photocell was recorded by the mirror-galvanometer (I) (Fig. 4.).

By measuring the source of light with current from an accu-

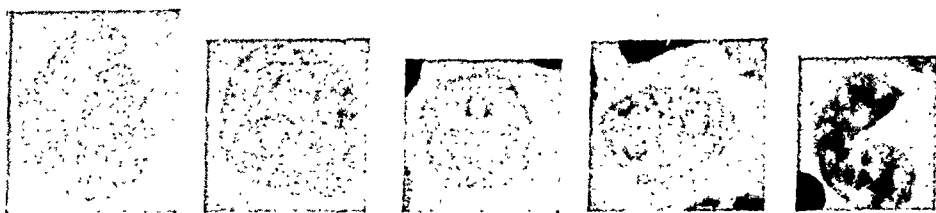


Fig. 6. Feulgen-stained myeloid cells. Observe the nucleolar circle.



Fig. 7. Feulgen-stained erythroid cells. Greatly condensed chromatin.

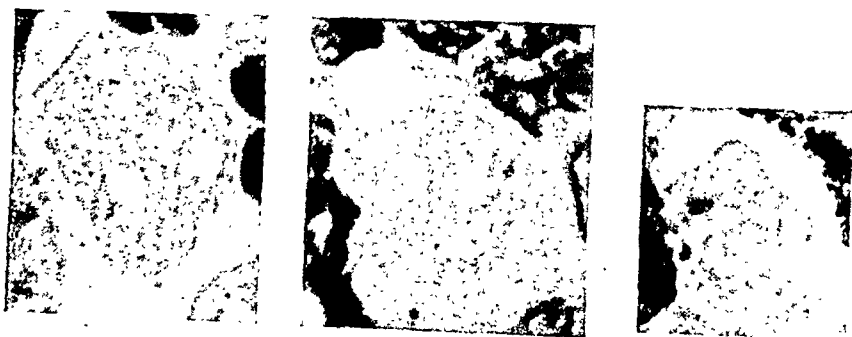


Fig. 8. «Doubly stained» granulocyte cells (see text). Particularly well suited for measuring the size of the cell organelles.

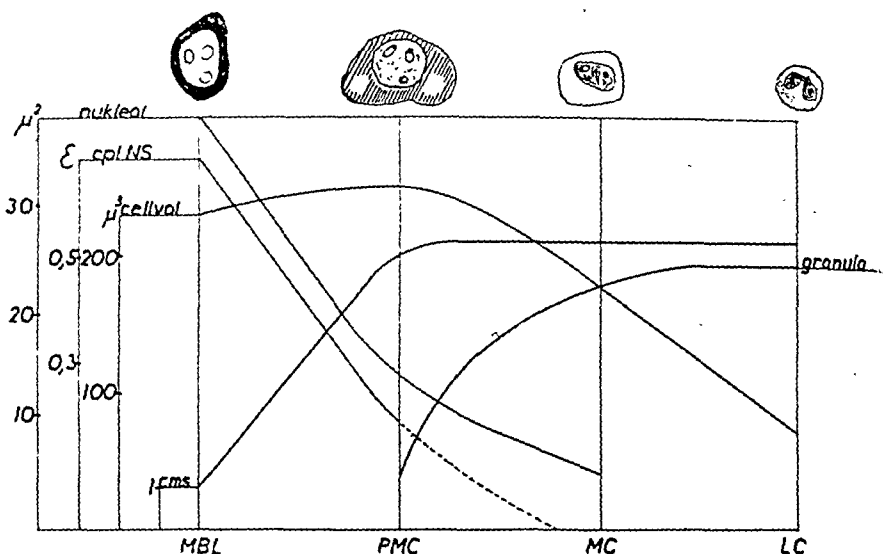


Fig. 9. Survey of some essential cytochemical and cytological data during the differentiation of a myeloblast into a mature granulocyte. Cpl NS = concentration of cytoplasmic nucleotides expressed in the coefficient of extinction. Nukleol = total nucleolar mass expressed in μ^2 . Cellvol = cell volume in μ^3 : cms = total volume of the different stages. Granula = the approximate granula content in each type of cell.

The outline drawing shows how, before and during the growth phase, the cytoplasm of the stem-cell (mb1) is packed with nucleotides, and how rapidly the concentration decreases in the course of differentiation, concurrently with a reduction of the nucleolar mass. In the later, relatively well-differentiated stages, where the nucleolus-cytoplasmic nucleotide mechanism is suppressed, the increase in the number of cells takes place at the expense of their size. The drawn cells are mainly intended to show the nucleus-plasma ratio and the nucleolus picture at different stages. In the primitive type of cell (mb1) the formation of a substantial nucleolar mass has broken up the nucleolus-associated chromatin into thin outer layer. Owing to the diminished formation of nucleolar substance by the nucleolus-associated chromatin during differentiation, the outer layer (cf Fig. 6) is gradually converted into a compact chromocentre.

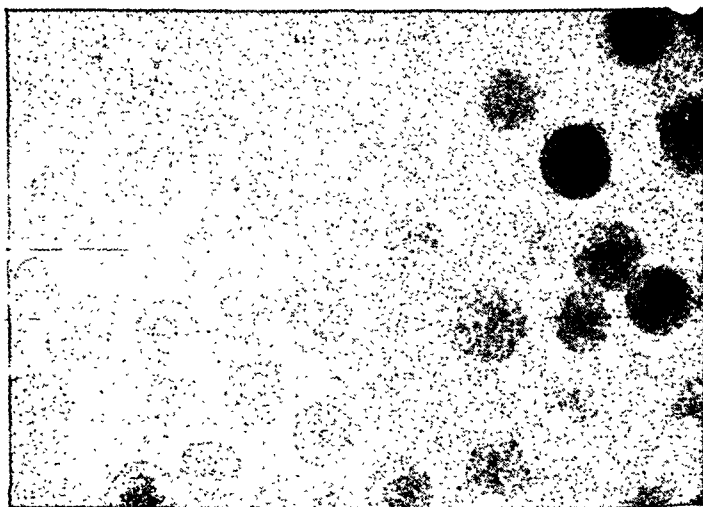


Fig. 10. Ultraviolet picture (2570 Å) of polychromatic and normal red cells. Marked difference in absorption.

ulator battery, fluctuations in the intensity of the light falling on the measuring were obviated.

For the values of the coefficients of extinction obtained ($\epsilon = \log I_0 - \log I_1$) of points measured in the cytoplasm, see fig. 5.

Localization of ribodesose nucleotides.

For the localization of ribodesose nucleotides in the cellular structures, the Feulgen nucleal reaction, as modified for blood cells by Honneth in 1940, was employed. With a view to the reliable determination of the different cells, the Feulgen-stained preparations were compared with those which, after such staining, had also been faintly stained with May-Grünwald's methylene blue-eosin.

The result of the treatment of cytological material with fuchsin sulphurous acid after acid hydrolysis has been found to be rather specific for ribodesose nucleotides (Feulgen and Rossenbeck 1924). Other substances which, after hydrolysis, have a free aldehyde group, and which occur in cytological material, are Feulgen's plasmogen, plasmal and other lipoid-aldehydes. On comparing the Feulgen reaction in sections or films treated with alcohol after acid hydrolysis, and in which the fatty-acid aldehydes had been dissolved, the same results were obtained as in the smear preparation. These substances therefore did not affect the cytological picture in the ordinary Feulgen-stained preparation.

The bone-marrow preparations consisted of smears produced by the cautious dabbing on a slide of separately prepared femur bone marrow from adult rats. The films were fixed with formalin fumes for one minute. *Preparations produced in this way, as compared with living cells photographed in ultraviolet light, showed very small changes in the cytological picture.*

In all the cells the chromatin network showed a rich stain. (Fig. 6 and 7). On the other hand, *the cytoplasm, which in the earliest stages was markedly basophil and intensely absorbed ultraviolet light, gave an entirely negative result to the Feulgen test.* This of course was likewise the case with the acidophil, granula-filled cytoplasm of the more or less differentiated cells.

The intensely absorbing nucleoli of the myeloblast, promyelocyte and early erythroblast were entirely Feulgen-negative. (Fig. 6) The

large nucleolus of the myeloblast was delimited by a thin, but quite marked, outer layer, which, in view of its distinct staining, evidently contained ribodesose nucleotides. In the later, more differentiated, stages the concurrently reduced nucleolar mass gradually acquired a thicker, Feulgen-positive, outer layer.

The reduction of the Feulgen-negative nucleolus was found to proceed continuously during the development of the cell (see below). The nucleolus of the myelocyte, for example, was observable merely as a small, thick, irregular ring and finally, in the metamyelocyte, was not developed at all, being reduced to a small, Feulgen-positive clump, which, according to Caspersson and Thorell (1942), is to be regarded as a chromocentre.

It is noteworthy that this degeneration of the nucleolus proceeds far more rapidly in the erythropoietic series than during the myelopoiesis (Fig. 7).

From a preliminary comparison between the appearance of the bonemarrow cells after the Feulgen reaction and on the ultraviolet microphotograph, it appears (1) that, as regards the cytoplasm, the intense ultraviolet absorption in the myeloblast and the early erythroblast is presumably derived from ribose nucleotides, and (2) that the large nucleoli of the same early stages contain a relatively high concentration of ribose nucleotides, the amount of which gradually diminishes as the differentiation proceeds, so that the intensely absorbing formations in the nucleus of later, more or less fully differentiated cells consist entirely of ribodesose nucleotides.

Micro-incineration.

From the ultraviolet photograph the intracellular distribution of nucleotides, through the purin and pyrimidin base of those substances, can be observed. These observations may be supplemented and confirmed by micro-incineration of the smear preparations.

Ordinary adult tissue has an ash content of about 2 per cent. Microincineration has shown that the major part of this ash as a rule is derived from the nucleus. In cells with at least 6 per cent. of nucleotides in the cytoplasm a corresponding increase of cytoplasmic ash may, however, be anticipated. In fact, owing to the

high content of phosphorus (about 25 per cent.) in the nucleic acids, cells containing large amounts of nucleotides may be expected to yield a copious amount of ash. At an incineration temperature of 500 Celsius the phosphates, which in these cells constitute the major part of the ash, are left. The distribution of the ash within the cell can easily be observed in the dark field illumination.

This correlation between the ultraviolet and micro-incineration pictures was shown by A. Engström (1943) on a large material.

Preparations thus incinerated showed (1) a high content of ash in the cytoplasm of the myeloblast and (2) naturally a copious residue of ash from the more or less condensed nuclei of the more differentiated cells.

In view of the coarseness of the ash picture and the small dimensions of the cellular structures, micro-incineration did not admit of a reliable examination of the finer organelles.

III. Discussion of the material.

1. *Does the nucleolus nuclear-membrane apparatus participate in the formation of cytoplasmic proteins during the adult hematopoiesis?*

As stem-cells for the granulocytes and erythrocytes cells of very similar cytological types (e. g. Maximow's hemocytoblast, Pappenheim's lymphoidocyte, Naegeli's macroblast,) have been described. This similarity of origin is indicated also by the ultraviolet pictures of the two stem-cells. Both are rather large, the cytoplasm intensely absorb ultraviolet light, the nucleus is round and contains several large, absorbing nucleoli. To judge by the Feulgen reaction, these nucleoli consist mainly of ribose nucleotides, surrounded by a ribodesose nucleotide layer. In the subsequent forms, however, great differences between the myeloid and erythroid cells, such as the marked disparity in the development of the cytoplasm, can be observed.

A value of the size of the cytoplasm in the different types of cell of the two series can be obtained by direct measurement. In computations made by the author, the average size of the cells photographed in ultraviolet light was taken as a basis. The magnification was 1150 \times . Artefacts which might have affected the

results were eliminated in so far that the cells were not dried, nor appreciably flattened. The following values, expressed in μ , were obtained:¹

	Diameter in μ		
Myeloblast	12.0 \pm 0.16	$\sigma = 0.7$	
Promyelocyte	14.4 \pm 0.15	$\sigma = 1.05$	
Myelocyte	10.1 \pm 0.11	$\sigma = 1.15$	

In order to check the above results, it seemed desirable to measure the size of the cells also in a stained preparation. A far larger number of cells can be measured in this way, which is of special importance as regards the less accessible undifferentiated stages. For this purpose preparations produced in accordance with the previously described technic and doubly stained with Feulgen and May-Grünwald's methylene blue-eosin are very well adapted. (Fig. 8) On such preparations the optical cross-sectional area of the aggregate nucleolar mass can be exactly measured. The cellular constituents in question were in fact clearly marked and distinctly delimited, without any visible shrinkage, or the like, whatsoever. The values thus obtained will be used in the following discussion.

Magnification: 1500 \times . Measuring instrument: the Zeiss ocular screw micrometer. With this optical apparatus, a precision down to measurements of 0.1 μ was easily attainable. The cell organelles in question were measured in a hundred cells of each type.

Mean values, mean error and dispersion.

	Cell diameter in μ			Area of nuelcolus in μ^2		
Myeloblast	16.8 \pm 0.1	$\sigma = 0.3$		38.8 \pm 0.10	$\sigma = 0.6$	
Promyelocyte	17.6 \pm 0.25	$\sigma = 1.5$		12.7 \pm 0.18	$\sigma = 1.06$	
Myelocyte	15.4 \pm 0.2	$\sigma = 1.1$		5.1 \pm 0.23	$\sigma = 1.2$	
Mature cells	9.5 \pm 0.18	$\sigma = 1.03$		—	—	—

If the mean diameter of the cells photographed in ultraviolet light is compared with that of the cells in the stained preparation,

¹ For the statistical treatment of the measurements the following methods have been adopted;

The dispersion is calculated in accordance with the formula $\sigma = \sqrt{\frac{\sum a^2}{n-1}}$, where a is the deviation from the mean value and n the number of observations.

The mean error of the arithmetical mean is calculated in accordance with the formula $e(M) = \pm \frac{\sigma}{\sqrt{n}}$.

we shall find throughout in the latter a rather considerable difference in size. The two series are of course not directly comparable, depending on different factors as for example the flattening in the stained preparation. But, as the object of this measurement is to obtain some idea of the *changes* in the size of the cells or cell organelles during the differentiation, a uniform disparity in the measurements obtained with the different methods is enough.

As regards the *erythroid* types of cell, the diameter was measured only in the cells photographed in ultraviolet light. The same precision as in the case of the myeloid cells cannot be attained here, as the transitions between the differentiation stages are rather fluid. But, as the values will be discussed only in connection with a comparison between the myeloid and erythroid series, such precision is scarcely necessary for the result. The following cell diameters were obtained:

Proerythroblast	11.5 μ
Basophil erythroblast	9.5 μ
Polychromatic erythroblast	7.5 μ
Orthochromatic erythroblast	6.5 μ

If the results of the measurements in the two series are compared, it will be found that the cells in the erythroid series, with the exception of the earliest cell, are marked by a relatively small amount of cytoplasm, as compared with the myeloid cells, in which a large cytoplasm is retained through almost the whole chain of differentiation.

Several authors (e. g. Downey) have described how the nucleoli, characteristic of the undifferentiated stages both in the erythroid and in the myeloid series, in the course of the first-mentioned maturation process, disappear at a very early stage, whereas in the latter process they are observed right up to the myelocyte stage.

The results of the cytochemical investigations reported above showed also that the nucleolar apparatus was very differently developed in the two series. In the myeloid series large nucleoli, containing ribose nucleotides were observed. This type of nucleolus did not entirely disappear until the myelocyte stage was reached.

From the fact that the cells in the myeloid series show large cytoplasm, whereas those in the erythroid series are comparatively small, it follows that the production of proteins during the

growth of the former cells proceeds with considerably greater intensity than in the latter. If we correlate with this fact the above described cytochemical pictures presented by the nucleolar nuclear-membrane apparatus in the two series, it will be found that the intense production of proteins in the myeloid cells runs parallel with an evidently well-developed nucleolar apparatus, whereas in the erythroid cells — in conformity with the far less marked formation of new cytoplasmic protein —, such an apparatus is developed only in the very earliest stages, and subsequently disappears.

The conclusion which must be drawn from this discussion is thus that the formation of cytoplasmic proteins, even during the adult hematopoiesis, proceeds with the aid of special organelles, namely the nucleolus and nuclear membrane, similarly as in the previously studied types of cell, for example, embryonal cells.

The blood cell regeneration.

A question of great importance for the discussion regarding the regeneration of the blood cells is whether the development of a blood cell into its mature form in the normal, adult animal starts mainly from the most primitive cells, such as the myeloblast, and proceeds by a series of heteroplastic divisions, or sets out from more differentiated stages, such as the myelocyte, thus proceeding chiefly by homoplastic divisions.

The view held by several investigators (e. g. Rohr 1939) is that the formation of new blood cells in the normal, adult animal proceeds in the main from relatively well-differentiated cells; that during embryonal life, on the other hand, differentiated elements develop from undifferentiated cell forms by heteroplastic division; and that in an adult individual such a direct origin of a mature blood cell from an undifferentiated cell is possible only under pathological conditions.

This view has resulted from the attempts made to determine the intensity of growth of the various differentiation stages. Such computations have been based on a differential count of all the mitoses in a section or smear preparation.

This different intensity of growth in the stages passed through during the maturation of the cell has also an important bearing on

the present investigation, as an elucidation of this question should render it possible to correlate the endocellular chemical processes in the various hematopoietic cells with the rapidity of growth and degree of differentiation in the corresponding stages. It seems therefore desirable to enter more fully into this question.

The above mentioned determination of the intensity of growth in the various differentiation stages is founded on the ratio between the number of mitoses of the different cells. (Specifically the ratio between the quotients — the number of mitoses: the number of cells.) It is questionable, however, whether this computation of the mitosis frequency (in sections or preparations) is a reliable method for determining what type of cell is most burdened with the blood-cell regeneration, and which consequently must possess the greatest capacity for growth. The conditions which must be fulfilled if this ratio is to serve as a reliable index of the intensity of growth is (1) that the mitoses should proceed at the same relative pace and (2) that the cells produced after the division should be of the same kind.

Otherwise (1) the rapidity of growth of a cell type with slowly proceeding mitoses will be estimated at too high a figure. That, as regards the hematopoiesis, such a source of error in all probability affects the estimate of the intensity of growth of the cellular constituents is indicated, *inter alia*, by La Cour's observation that cells passing into the polynucleated form have a suppressed anaphase.

The second factor in computing the intensity of growth according to this method, namely the number of the cell type in question in percentage of the total types of cell, likewise involves an indeterminable source of error. The essential point is whether the mitosis of a cell proceeds by heteroplastic or by homoplastic division, which determines whether the cells produced after the mitosis are retained within the cell group or not. Thus in the case of a homoplastic mitosis a lower figure will be obtained for the intensity of growth, and vice versa.

This method of computing the intensity of growth in the different stages through which the blood cell passes during maturation seems therefore not to be entirely reliable.

It was indicated in the introduction that it would be possible to compute the state of function of the cytoplasmic protein-forming

system of the individual cell by studying the state of development of the organelles participating in the formation of cytoplasmic protein (the nucleolus and nuclear membrane) as well as the content in the cytoplasm of ribose nucleotides formed from the nuclear membrane.

From the above comparison (p. 354) between the appearance of the bone-marrow cells after the Feulgen reaction and on the ultra-violet microphotograph, it was seen that the development of the nucleolar nuclear-membrane mechanism pointed to an intensive protein-forming function in the most undifferentiated stages, such as the myeloblast or proerythroblast, whereas in later stages, such as the myelocyte or basophil erythroblast, it showed signs of a far weaker function. The earlier forms in the chain of differentiation are thus engaged in more intensive proliferation than the more mature stages. From this it may be inferred that the development of the adult animal's mature blood cells, intended to cover the daily requirements, begins, at any rate to a large extent, in the most primitive forms and is continued by a series of *heteroplastic* divisions.

Having thus assumed — as seems quite legitimate —, that the regeneration proceeds mainly by a series of heteroplastic divisions, it will be possible from other, more precisely determinable data, to obtain some idea of the intensity of growth of the different stages.

Since the composition of the hematopoietic organ, as regards the different types (stages) of cell is fairly constant, their capacity for growth may be expressed as *the increase in cellular mass which takes place during the transition from one type of cell to a more differentiated type*.

As previously mentioned, the myeloblast serves as a stem-cell for the heteroplastically developing myeloid elements. The growth which takes place when increasingly mature cells are differentiated from such a stem-cell is naturally reflected firstly in an increase of the number of differentiated cells relatively to the stem-cell, and secondly in a modification of the size of the cells after the mitoses.

According to Stasney and Higgins, who have made very thorough differential counts on the bone marrow of the femur from white rats, the percentage distribution of myeloblasts avera-

ges 1.3, that of promyelocytes (plus leukoblasts) 8.6, that of myelocytes (plus metamyelocytes) 12.5, and that of still further differentiated cells 33. The remainder is the percentage of erythroid cells. From these data we can deduce the increase in the number of myeloid cells: from myeloblast to promyelocyte 6.6 times the number, from promyelocyte to myelocyte 1.5 times, and from myelocyte to mature granulocytes 2.6 times.

If we examine the mean diameter of the different types of cells in preparations, it will be found to be about $17\ \mu$ for the myeloblast, $18\ \mu$ for the promyelocyte, $15\ \mu$ for the myelocyte and $7\text{--}12\ \mu$ for the later stages. The volume of the cells in the smear preparation may approximately be reckoned as the volume of a cylinder with the base = the cell area and the height h . The slight difference in the height of different cells in a preparation may be set aside.

According to this computation, the volume of the myeloblast figures out at $230. h\ \mu^3$, of the promyelocyte at $250. h\ \mu^3$, of the myelocyte at $177. h\ \mu^3$ and of the mature cells at about $70. h\ \mu^3$.

The increase in the *total* cell volume in passing from one type of cell to a more differentiated one can be obtained from these values by multiplying the ratio between the volume of the more differentiated cell and the volume of the preceding cell by the increase in the number of cells. The increase in the total volume between the myeloblast and the promyelocyte thus figures out at 1: 7.2, between the promyelocyte and the myelocyte at 1: 1, and between the myelocyte and a fully differentiated cell at 1: 1.0, or, rounded off, 1: 7: 7: 7.

If the formation of granulocytes is supposed to proceed heteroplastically, these figures show that an increase in cell volume takes place almost exclusively at a very early stage of differentiation. *In later stages, where the growth is terminated, the divisions take place at the expense of the size of the cell.*

Changes in the cytochemical picture during the maturation of the blood cell.

A. Myeloid series.

The above described ultraviolet photograms of cells belonging to the myeloid series show in the most undifferentiated cells, namely the myeloblasts, a high content of cytoplasmic nucleotides:

As the Feulgen test gave a negative result, these nucleotides are evidently of ribose character. During the differentiation of the myeloblast into the mature granulocyte, the concentration is considerably reduced: even in the transition to the promyelocyte type, the concentration, expressed in the coefficient of extinction for the cytoplasm at 2570 Å, has fallen to half. Owing to the increasing interspersation of refractive granula in the cytoplasm during maturation, this diminishing content of nucleotides cannot be followed with the same precision in later stages. Other cytochemical data, however, point to a continuous diminution during differentiation.

As the increase in cellular volume was largest between the myeloblast and the promyelocyte, this observation tallies well with the results of previous investigations (Caspersson and others) that cells in intense growth are characterized by high concentrations of nucleotides in the cytoplasm. The longer the cell is differentiated and the less its need of growth, the less will be this concentration of cytoplasmic nucleotides.

Viewed in conjunction with observations on other material (Caspersson, Schultz, Thorell and others), this indicates that these nucleotides take part in the formation of new cytoplasmic protein.

The results obtained in staining the cytoplasm indicate a continuous diminution of the amount of acid groups during differentiation: earlier stages, such as the myeloblast, have a markedly basophil cytoplasm, and this basophilia, during the transitions from the promyelocyte, through the myelocyte, to the granulocyte, continuously diminishes in inverse proportion to the granula formed during differentiation.

Of particular interest is the cytological observation made by several authors (references in Downey 1932) that in a rather large number of myeloblasts there is a perinuclear, acidophil area. This indicates that in these cells, in the immediate vicinity of the nuclear membrane, there is a preponderance of basic groups. According to earlier references (Caspersson and Thorell) it was shown by absorption analyses that, in addition to ribose nucleotides, proteins rich in hexone bases, emanating from the nucleolus, also occurred during the building-up of cytoplasmic protein. The inconstancy of the staining picture, that is, the homogeneous basophilia of the cytoplasm in some of the myeloblasts, need merely signify that the acid

groups in the nucleotides preponderate relatively to the proteins rich in hexone bases.

The cytochemical changes which the nucleus undergoes are the following: —

In the myeloblast the nucleus is large, has a fine-grained chromatin structure and contains an ample nucleolar mass (optical cross-sectional area = $39 \mu^2$). During maturation the nucleus diminishes in size, likewise the nucleolar mass, whereas the amount of cytoplasm does not undergo any noteworthy change in size. The relation between these nucleolar changes, reductions in the amount of cytoplasmic nucleotides, intensity of growth and degree of differentiation are illustrated in Fig. 9.

During the development from the myeloblast to the mature granulocyte, a condensation of ribodesose nucleotides in the nucleus takes place concurrently with the diminution of the nucleolar substance (see the Feulgen picture). The content of ribose nucleotides in the cytoplasm diminishes continuously, simultaneously with an increase in the amount of granula. In the first stage of development, the cell volume shows a small increase, but afterwards, according as the growth mechanism is brought to a standstill, the cells produced after mitosis become smaller and smaller. Granula probably arise by the conversion of the original proteins in the cytoplasm, and thus the amount of fundamental cytoplasmic protein becomes still smaller than corresponds to the size of the most fully differentiated cells.

B. Erythroid series.

The earliest stage, the proerythroblast, has, broadly speaking, the same characteristics as the myeloblast: a large nucleus with a diffuse content of ribodesose nucleotides. In the nucleus there are one or two nucleoli. The correlation between the ultraviolet picture and the Feulgen staining shows that the nucleolus contains large amounts of ribose nucleotides. The cytoplasm is relatively scanty and likewise contains a considerable concentration of ribose nucleotides.

The ultraviolet pictures of the whole following series show that those parts of the nucleus which contain ribodesose nucleotides become more and more compact according as the nuclear volume

diminishes. *The nucleolus containing ribose nucleotides disappears at a very early stage in the differentiation.* All that is left is a marked area containing ribodesose nucleotides, which, according to Caspersson and Thorell, is to be regarded as a chromocentre. Parallel with the differentiation, the concentration of cytoplasmic nucleotides likewise diminishes. At the same time a formation of hemoglobin takes place in the cell, presumably by the conversion of the cytoplasm's own proteins.

The above cytochemical changes are precisely reflected in the staining picture. The basophilicly stainable ribodesose nucleotides in the nucleus are arranged at an early stage at rather large intervals, but in the course of differentiation they gradually clump together, so that an extremely basophil chromatin clump finally remains. In the undifferentiated proerythroblast, the nucleolus contains mainly proteins rich in hexone bases and ribose nucleotides, the former of which entirely obscure the acid groups of the nucleic acid (Hammarsten and Teorell 1928), whence an affinity for acid dyes results. The reverse is the case on the ultraviolet picture, where the nucleotides preponderate by their high absorption at 2600 Å. — In later stages the acid groups of the ribodesose nucleotides of the nucleolus-associated chromatin predominate in increasing degree, whence in the more differentiated stages they can be distinguished from the remaining chromatin by a largish basophil clump, the chromocentre.

The cytoplasm in early stages, owing to its high content of ribose nucleotides, is intensely basophilic. In the course of the differentiation, with diminishing capacity for cell growth, the nucleotide concentration is reduced and the cytoplasm becomes less basophilicly stainable. At the same time the amount of acidophil hemoglobin increases.

A basophilia may, however, persist in erythrocytes circulating in the blood, as is the case with the so-called polychromatic erythrocyte. On examination in the ultraviolet microscope with monochromatic light corresponding to the absorption maximum of the nucleic acids, these erythrocytes are found to show an absorption which in all probability corresponds to a considerable amounts of nucleotides (Fig. 10). As the Feulgen test was entirely negative, these nucleotides are probably of the ribose type; it may therefore be presumed that, despite the absence of a nucleus, these poly-

chromatic blood cells, in view of the chemical composition of their cytoplasm, have not reached a fully differentiated stage.

Changes in the chromocentre-nucleolar apparatus during the maturation of the blood cell.

The most undifferentiated stage, the myeloblast, which was characterized by its high capacity for growth, had a distended nucleus with large nucleoli containing ribose nucleotides. In the cytoplasm large amounts of ribose nucleotides had collected. Thus this undifferentiated cell, in which a rapid formation of cytoplasmic proteins was proceeding, showed a cytochemical picture which clearly indicated a nucleolus-associated chromatin and nucleolar system in intense operation. A similar type is the growing egg-cell and the rapidly dividing embryonal cell.

By using living cells for the ultraviolet picture, and by treating the Feulgen-stained preparation in an extremely cautious way (fixation with formalin fumes), such good cytochemical pictures could be obtained that the development of the nucleolus during differentiation could be followed in detail without difficulty.

On the Feulgen-stained preparation one could very clearly observe the annular accumulation round the nucleolus of ribodeoxy nucleotides, which, according to previous investigations, are to be regarded as a chromocentre (Caspersson and Thorell 1941). This formation is termed, e.g. by Schridde, »Eimer's nucleolar membrane». The probable explanation of the annular arrangement is that, when the chromocentre during the interphase forms ribose nucleotides and proteins rich in hexone bases, these substances, according to their amount, more or less disrupt the heterochromatic area. If the cell after mitosis is not to grow in any appreciable degree, as is the case e. g. with the myelocyte, the polynucleotide system of the cytoplasm is developed merely to a small extent for protein-formation. As, parallel with this, the nucleolar substance is not very abundant, the surrounding layer containing ribodeoxy nucleotides has a smaller diameter.

In connection with the differentiation into the mature granulocyte, the undifferentiated, embryonal types gradually disappear. This is correlated with the diminishing capacity for growth characteristic of the myeloid cell during maturation (see below). From

the myeloblast to the promyelocyte stage, cells of this type were capable of increasing their total mass 6 to 8 times, whereas in later stages this larger mass remained practically constant.

The most primitive cell in the adult hematopoietic series, the myeloblast, contained in its nucleus two to four round formations, which cytochemical analysis showed to contain considerable amounts of ribose nucleotides. These nucleotides were surrounded by Feulgen-positive substance (the nucleolus-associated chromatin). The nucleus was rather large and rich in evenly distributed euchromatin. The cytoplasm was small relatively to the nucleus, that is, the nucleus-plasma ratio was high (0.7).

In the next stage the nucleus had diminished in size, though the entire cell body in this stage was larger than in the preceding one (a lower nucleus-plasma ratio, namely 0.5). The nucleolar mass had diminished considerably, and the nucleolus was observable as a more or less irregularly round formation, but still containing ribose nucleotides. The surrounding nucleolus-associated chromatin, in correspondence with the reduced nucleolar mass, was less disrupted and thus appeared as an irregular, rather thick ring.

In the myelocyte, whose cytoplasmic protein had been mainly converted into granula and had lost most of its basophilia, the nucleus was small, the euchromatin clumped together, and the extremely small nucleoli were surrounded by a thick layer of nucleolus-associated chromatin.

The mature leukocyte, which has no function subserving growth, had a markedly differentiated, lobed nucleus. No nucleoli had been formed: the nucleolus-associated chromatin (the chromocentre) lay inert and in an irregular Feulgen-positive clump.

The relation between these types of nucleolus, those of the myeloblast, the promyelocyte and the myelocyte, is most simply explained by the supposition that the nucleolus-associated chromatin (the chromocentre) in the different stages had formed varying amounts of ribose nucleotides.

Caspersson's studies of salivary-gland nuclei from *Drosophila* had shown that the chromocentre and the nucleolus both contained substances the absorption spectra of which indicated the presence of proteins rich in hexone bases. This, in conjunction with other circumstances, makes it probable that the major part of the nucleolar substance had been formed from the nucleolus-

associated chromatin. As the nucleolus also contained ribose nucleotides, its affinity to acid or basic dyes will be determined by the relation of these substances to one another. Most of the nucleoli in the undifferentiated cells (except the annular Feulgen-positive formation, regarded as a chromocentre) contained ultra-violet-absorbing, Feulgen-negative ribose nucleotides. By the production of these nucleotides the chromocentre was gradually disrupted, and is recognized on the Feulgen- and Giemsa-stained preparation as a Feulgen-positive, basophil circle round the nucleolus. As this nucleolus on a hematoxylin-eosin preparation stained acidophilicly (Butterfield and others), this must be due to the presence of basic groups, probably derived from proteins rich in hexone bases. The amount of these proteins cannot be stated, as even quite small amounts of protein may completely obscure the acid groups of the nucleic acid in the staining (E. Hammarsten, G. Hammarsten and T. Teorell 1928). The development of the nucleoli of the hematopoietic elements, in conjunction with the high content of ribose nucleotides in the cytoplasm in the undifferentiated stages where growth is proceeding, thus agrees well with the previous observations made regarding the development and function of this cell organelle.

Embryological and genetic data have shown (Caspersson and others) that the protein-forming system of the cell, and thus its growth, is regulated by a combination of factors, cytochemically defined as nucleolus-associated chromatin (see p. 338). *During maturation, via the above-mentioned characteristic cell types, the activity of the nucleolus-associated chromatin — nucleolus — cytoplasmic-nucleotide system continuously diminishes, which results in a reduced new formation in the cell of cytoplasmic protein.* This phenomenon may also be expressed by stating that the more a cell is differentiated for its final function, the more it loses its capacity for growth. The cause of this phase in the differentiation process must thus be sought in the nucleolus-associated chromatin.

A certain periodicity in the function of this chromatin is shown during different stages of interphase and division. During mitosis the nucleus shows a marked increase of ribodesose nucleotides. The nucleolus has completely disappeared and thus, of course, plays no part in the formation of cytoplasmic nucleotides. During mitosis the factors which mainly determine the function of the cell, namely

the genes, are concentrated in the chromosomes, rich in nucleotides. As Caspersson and Schultz have shown, the heterochromatic chromosome parts during mitosis serve to regulate the nucleic acid metabolism of the chromosomes. Between the divisions the nucleolus-associated chromatin, through the nucleolar-cytoplasmic-nucleotide system, will regulate the growth of the cell. This is effected by the production of nucleolar substance in the form of ribose nucleotides and proteins rich in hexone bases.

The explanation of the reduction of the capacity for growth, proceeding parallel with the differentiation, may thus either be a diminution of the activity of the nucleolus-associated chromatin, or else a decrease in its amount. As this chromatin, unlike the euchromatin, is built up of identical or similar gene elements (Caspersson 1941) a decrease in amount in principle is conceivable. For other reasons, however, a decrease in amount does not seem probable in this case.

Nevertheless certain changes in the amount of the heterochromatin have been observed during the gene division (Schultz and Caspersson 1939, 1940). Examples are also known where the sex cells contain large amounts of chromatin which is eliminated in the somatic cells and which seems to have heterochromatic character, as for instance in *Ascaris*.

Whether the reduced activity of the nucleolus-associated chromatin during the differentiation is due to such a diminution in amount cannot be determined with certainty. That such elimination should occur in any appreciable degree is, however, improbable, seeing that in relatively well-differentiated cells, such as the myelocyte, one observes round the poorly developed nucleoli a considerably thicker layer of Feulgen-positive nucleolus-associated chromatin than in the earlier forms, where this layer was extended by abundant nucleolar substance. It should be noted, however, that the Feulgen picture does not show any precise quantitative result.

It is interesting to compare the nucleoli in different phases of the differentiation of the hematopoietic cells with the nucleoli occurring in cells in different layers of a cancer cord (Caspersson and Santeson 1942). The authors referred to consider it probable that the rapid growth in malignant cells is due to a pathological hyperfunction of the nucleolus-associated chromatin. The differentia-

tion between an outer cell (A type) and an inner cell (B type) is due, according to those investigators, to the poor nutrition in the centre of the cancer cord.

The nucleoli of the »differentiated» B type are large and still contain considerable amounts of ribose nucleotides. The nucleolar development of the differentiated granulocyte, on the other hand, is quite suppressed. The difference between this »false differentiation» from the A to the B type and the differentiation from the myeloblast to the granulocyte will then be realized: in the former case the nucleolus-associated chromatin is throughout supernormally active and the »differentiation» into the poorly growing type is caused by directly operating external factors, whereas in the latter case during the hematopoiesis a quite normal differentiation is presumably taking place, because the primary stimulus proceeding from the environment is in operation through the nucleolus-associated chromatin.

IV. General Summary.

Earlier investigations have shown that the formation of cytoplasmic proteins during the growth of a cell generally proceeds with the aid of certain cell organelles, more especially the chromocentre, nucleolar apparatus and nuclear membrane. From the state of development of these organelles, especially the amount and composition of the nucleolar material, as well as the content in the cytoplasm of ribose polynucleotides formed from the nuclear membrane, *it has been found possible to determine the state of function of the cytoplasmic protein-forming system of individual cells, (their intensity of growth).*

The results of a study of the development of this system in the blood-forming organs of adult mammals, with the aid of certain cytochemical methods, are recorded in this paper. The problem set was (1) to investigate whether the cytoplasmic protein-forming system in these cells operates on the same lines as in other previously studied types of cells and (2) to utilize the possibilities of determining the state of growth of individual cells in order to settle certain questions regarding the formation of the blood cell in bone marrow. One of the principal questions was whether the blood-cell regeneration in the adult organism mainly starts from the most

primitive stem-cell and proceeds by a series of heteroplastic divisions, or whether it sets out from more differentiated stages and proceeds by homoplastic divisions.

The living cells from the bone marrow of rats were examined with ultraviolet microscopy and a simplified absorption method for the purpose of judging the content of polynucleotides in the cytoplasm. The results of these methods were checked with the Feulgen reaction, micro-incineration and staining with acid or basic dyes, the last-mentioned procedure being specially intended for studying the development of the nucleolar apparatus.

Granulocytes and erythrocytes are derived from cells of very similar cytological types. In the cell generations succeeding the stemcell the two series are distinguished, *inter alia*, by the marked difference in the development of the cytoplasm. The erythroid series, with the exception of the first daughter-cell, is marked by a very slight development of the cytoplasm, whereas the cytoplasm of the myeloid cells are well developed, which was shown by direct measurements of size. *These investigations indicate that the degree of development of the nucleolar nuclear-membrane apparatus is quite different in the two series.* In the cells of the myeloid series large nucleoli containing ribose nucleotides as well as considerable amounts of cytoplasmic nucleotides are observed. In the cells of the erythroid series, on the other hand, distinct nucleoli are observed in the very earliest stages and subsequently disappear. The largish rounded bodies in the nuclei of these later stages have not the character of true nucleoli, but must be regarded as chromocentres.

If the different pictures which the nucleolar nuclear-membrane apparatus presents during the development of the two series are judged according to the above-mentioned earlier observations on other cell material, they signify that in the cells of the myeloid series the formation of cytoplasmic protein proceeds with great intensity, whereas such protein is formed merely to a slight extent in the erythroid cells. This is in complete accord with the fact that the former have large cytoplasm and the latter merely small ones. *Thus in these cells too the cytoplasmic protein-forming system seems to operate on similar lines as in the previously studied types of cell, e. g. embryonal cells.*

The question whether the hematopoiesis in the adult animal

mainly starts from the most primitive blood cells and proceeds by a series of heteroplastic divisions, or whether it sets out from later stages in the chain of differentiation, thus in the main homoplastic divisions, has been much discussed. Several investigators have attempted to judge the intensity of growth of the different types of cell by counting the number of mitoses occurring in the different types. It is shown here that, for various reasons, the intensity of growth cannot be reliably estimated in this way.

The study of the development of the nucleolus nuclear-membrane apparatus in the different types of cell pointed to *an intensive functioning of the cytoplasmic protein-forming system in the myeloblast and promyelocyte, and to a marked diminution of that activity in later stages* (myelocyte and metamyelocyte). The earlier forms in the chain of differentiation were thus engaged in intensive proliferation. From this it follows that, in the adult animals examined, the hematopoiesis, at any rate to a large extent, begins in the most primitive forms, and thus proceeds by a series of *heteroplastic* divisions. Whether divisions of homoplastic type also occur could not be determined.

The total volume of cells belonging to different stages of differentiation was computed firstly by measurements of size in ultra-violet pictures and smear preparations, and secondly by differential counts of cells in bone marrow. It was shown that in the myeloid series the volume of the promyelocytes far exceeded that of the myeloblasts, and that the cell volume in the stages following the promyelocyte kept fairly constant. This indicates that the granulocytogenesis proceeds mainly from the myeloblast and that after the promyelocyte stages but little growth takes place. As above mentioned, the activity of the nucleolar apparatus shows a rapid reduction in these last-mentioned stages.

A thorough study of the cytochemical pictures showed that the typical appearance of the cells in the different stages was due to the continuous decline in the activity of the nucleolar apparatus. In the earliest form of cell, the myeloblast, large nucleoli, containing ribose nucleotides, had developed from the chromocentre and had disrupted the nucleolus-associated ribodesose nucleotides so as to reduce them to an annular formation surrounding the nucleolus. In the immediately succeeding type, the promyelocyte, the nucleolar mass had been reduced to about half the size. In connection

with this, the surrounding ring of nucleolus-associated ribodesose nucleotides was less disrupted and had more irregular outlines than the preceding cell. The diminution of the nucleolar mass proceeded continuously during the differentiation, so that the nucleolus of the myelocyte was very small and surrounded by a thick, irregular Feulgen-positive ring. In the metamyelocyte no nucleolar mass was developed at all: the larger, rounded formation in the nucleus consisted mainly of ribodesose nucleotides.

As above stated, a similar reduction of the nucleolar mass was observed also in the erythroid series, but with the difference that it proceeded with much greater rapidity, relatively to the maturity of the cell.

Parallel with this diminution of the nucleolar mass, the concentration of ribose nucleotides in the cytoplasm was reduced. Thus, during the development of the myeloblast into the pro'myelocyte, the concentration, expressed in the coefficient of extinction in 2573 Å, decreased from 0.7 to 0.3. These cytochemical changes could be correlated with a diminishing intensity of growth in the blood cells, concurrently with the differentiation. This is shown in outline in Fig. 9.

The marked acid properties of the substances whose changes in concentration could be followed with the cytochemical methods employed serve to explain the cytological pictures observed on an ordinary bonemarrow preparation, stained with acid and basic dyes. Thus the diminishing ability of the cytoplasm, during the differentiation, to bind basic dyes is due to the decreasing concentration of ribose nucleotides. The large nucleolus, containing ribose nucleotides, which is characteristic of the early stages where the cell is in process of rapid growth, has, however, a typical affinity for acid dyes; as previous investigations have shown, this is probably due to its content of proteins rich in hexone bases, which completely obscure the acid groups of the nucleic acid.

During the maturation of the cell, however, the acid groups of the nucleolus-associated ribodesose nucleotides predominate in increasing degree, so that in the nuclei of the more differentiated stages, these groups can be distinguished from the remaining chromatin as a large, rounded, basophil formation, a chromocentre.

Finally, the author discusses the primary cause of the continuously proceeding changes in the function of the blood cell during

differentiation, from the activities which serve the purpose of growth to the special function of the mature cell. The changes, during differentiation, in the organelle which plays a leading part in the cytoplasmic protein-forming system, namely the nucleolus, were explained by the fact that in the different stages the nucleolus-associated chromatin had formed varying amounts of *inter alia* ribose polynucleotides.

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Can Rheumatic Infection be influenced by an artificial Tropical Climate?

By

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Is the rheumatic infection¹ a climatic disease? At first sight the question may seem surprising. When speaking of climatic diseases we have accustomed ourselves to think in the first place of certain diseases in the tropics, possibly also of mountain sickness and certain other rare forms of disease occurring in quite special types of climate such as high-alpine. But it has been less usual to use this term for diseases in whose origin the climate of the temperate zones could be regarded as accessory.

A closer study of the influence of the climatic factors and weather on the origin and frequency of certain diseases and syndromes will show, however, that just as we may consider the tropical climate to be implicated in the prevalence of certain diseases and epidemics in these tropical climes, so may we with equally good reason regard our temperate climate as accessory to other ravages of disease. *Rheumatic infection as wide-spread disease in the population belongs to the climatic diseases of the temperate zones.* For if the areas over which rheumatic infection is spread are studied, considering in the first place the purest and the most easily demarcated of the clinical pictures, rheumatic fever, the frequency and appearance of which have been studied and analysed most in various parts of the world, we soon find that the areas in which

¹ A definition of what is here meant by rheumatic infection can be found in *Ergeb. inn. Med. u. Kinderh.* 52. s. 452 et seq.

it occurs in great frequency lie exclusively within the temperate climate, i. e. the temperate zones and those parts of the highlands of the tropics that have a similar climate.

Very early, Hirsch was able to demonstrate that rheumatic fever was very rare in the tropics. According to his handbook on geographical pathology there are certain exceptions, however, e. g. the uplands of Abyssinia according to Courbon, the tablelands of Arabia according to Pelgrave and Guez, and the high plains of Peru according to Mantegazza, i. e. regions within the tropics with a highland climate rather than a tropical climate in the strict sense.

Hirsch's observations have since been confirmed by a very large number of authors, among them Andersson, Buchanan, Tertius Clark, Clarke, Coburn, Getz, Newsholme, etc.

During 33 years' work as a physician in the Malay Archipelago Tertius Clark saw about 150,000 hospital cases and found among them not a single case of rheumatic fever, chorea minor, or mitral stenosis («the scarred valves of rheumatic disease»). In the Fiji Islands Clarke in 1930 found four cases of febris rheumatica, corresponding to 0.03 % of the total number of cases. Mangalik and Scott, during the years 1930—36, observed nine cases of rheumatic fever at the Thomason Hospital in Calcutta, these being 0.1 % of the medical cases of this institution. Andersson found only five cases of rheumatic fever or chorea minor among 3,000 internal hospital cases in Hong-Kong.

A number of patho-anatomical investigations have been undertaken to clarify the situation. In about 600 consecutive autopsies in Amritsar in India Keates was in no case able to detect any rheumatic affection or any vitium of the mitralis. Lambert and Pappenheimer had the same experience in about 500 consecutive postmortem examinations at San Juan in Porto Rico. In an autopsy material of about the same size in other islands of the West Indies Koppisch was able to find only two cases. Among 1,000 autopsies in San Juan carried out consecutively up to May, 1941, Carrillo found only eleven cases with affected mitral valves of probably rheumatic etiology. According to Heanley, no case was encountered of mitral stenosis or other undoubted rheumatic affection in over 6,000 autopsies in Hong-Kong. Pestana, at 793 autopsies in Singapore, found only thirteen cases in which the

mitral valves were affected. In Hughes, however, there is a statement that he had received at second hand that in 200 autopsies in Lahore, India, lesion of the mitral valves had been found in seven cases. It should be remembered, however, that a small portion of the districts taken in by Lahore and Amritsar consists of mountainous regions to the north, on the slopes of the Himalayas, which have not a tropical climate in the true sense.

Büngeler's investigation in Sao Paulo, Brazil, must be viewed from the same angle. He states that among 604 autopsies performed in this town during the period 1936—39 he found no fewer than 54 cases of rheumatic affections and that there were 29 such among 207 microscopically examined cases of this kind from the years 1940—41. Sao Paulo, it must be remembered, although situated not far to the south of the tropical circle, stands at a height of over 800 metres above the sealevel. Büngeler also points out the difference in respect of both morbidity and climate between Sao Paulo and the not far-distant town of Santos, which has a really tropical climate.

An investigation carried out by Getz in Panama is also worthy of mention, facilitating as it does the assessment of certain reports in the literature. Among 28,702 hospital cases in this town during a series of years he found 110 cases of acute febrile articular rheumatism, a very small percentage in itself. On making a careful clinical analysis of these cases he found, however, that no undoubted rheumatic affections could be demonstrated in any of them and that most of them consisted of tropical infections with joint-symptoms.

In subtropical regions, too, rheumatic infection is comparatively rare, and in any case is not an wide-spread disease to the extent it is in the subpolar halves of the temperate zones. Maxwell found that rheumatic fever did not occur except in very rare cases within the subtropical portions of South China, although it was to be met with in the more northerly parts of China, appearing in greater frequency the further north one came. Nicholl found only sixteen cases of rheumatic fever or chorea minor among 16,200 hospital cases in Miami, Florida.¹

An archeological study by Jones is interesting. He found not a single case of arthritic changes — though certainly numerous

¹ See also Table 4 on p. 55 in Edström: *Febris rheumatica*. Lund, 1935.

arthroses — among 35,000 Egyptian mummies examined by him. Bigelow and Lombard mention, however, that arthritic changes are reported to have been found by other investigators of Egyptian mummies.

Another old medical observation is that many severe cases of rheumatism recover if they are removed from the subpolar halves of the temperate zones to subtropical or tropical regions. This observation dates all the way back to Hippocrates and thenceforward can be followed through the times.¹ During the twenties and thirties of the present century Belgium, Great Britain, France, Holland, the U. S. A., as well as other countries with an unfavourable climate from a rheumatic point of view but with colonies situated within the tropics, have made practical use of this observation by establishing convalescent and sick homes for those suffering from rheumatism.

A most careful medical study of some severe cases of rheumatic fever with frequent recrudescences, which had been transferred from the unfavourable climate of New York to Porto Rico, was made about ten years ago by Coburn. He found that the extremely malignant rheumatic process in all of the ten cases followed by him during their six months' stay in the tropics came a standstill. After their return to New York, however, some of the cases had a relapse. Coburn's final therapeutic results were: four permanently recovered, one of which had however remained in Porto Rico, six had a relapse, three of which severe and three mild. Of these first three, two died and one relapsed into as severe a rheumatic condition as before. Of those second three, one returned to Porto Rico and was permanently restored, the other two also recovered from their relapse but no reliable information as to their subsequent fates has been available.

Coburn's most interesting observation was that the bacterial flora in the throat of these patients underwent a change during their sojourn in the tropics. During the last week in New York, before their departure to Porto Rico, cultivations from the throat disclosed hemolytic streptococci in all cases but one, these cocci

¹ See also Edström: Studies in natural and artificial atmospheric electric ions. *Acta Med. Scand.*, Suppl. 61, Lund, 1935, p. 1 ff.

being in abundance in eight cases. Three months later in Porto Rico Coburn found hemolytic streptococci in only one of all the cases, and even in this case these streptococci could only be sparingly detected during the last week in Porto Rico. After return of the patients to New York hemolytic streptococci recurred in five of the cases, and also though more uncertainly and temporarily in a sixth.

These observations gave birth to the idea that by means of an artificial tropical climate the same favourable effect might be achieved in rheumatic patients as the natural tropical climate had been found to exercise. The regulation of the climatical factors in a room or a house on a tropical pattern offers no great difficulties with modern air conditioning, especially if attention is confined in the first place to regulating the temperature and humidity of the air. There are, however, many different types of tropical climate — tropical primeval forest climate, most resembling our Russian baths, with almost 100 % relative humidity, tropical coastal climate, tropical continental steppe climate, tropical desert climate with, on the other hand, very low relative humidity — but also other variations. What is common to them all is the comparatively high temperature of the air, the relative absence of cyclones and anticyclones with little climatic variability, and considerable but regular diurnal variations.

The tropical chamber — or, as it should rightly be called, the climatic laboratory — was accordingly constructed so that (1) the temperature of the air could be adjusted between what is normal outdoor temperature with us and 40° C., (2) the relative humidity of the air could be adjusted between 30 % and complete saturation, and (3) both these factors could be kept constant at the level desired with a very small range of variation (for air temperature about 1° C and for relative humidity about 10 %).

The design of the chamber will be clear from Fig. 1. An ordinary sick-ward with space for two beds has been provided with double walls, double ceilings and double floors with free air space between the two layers. The internal surface of the old walls, ceiling and floor has been lined with insulating material. The old double-window has been furnished with another two inner

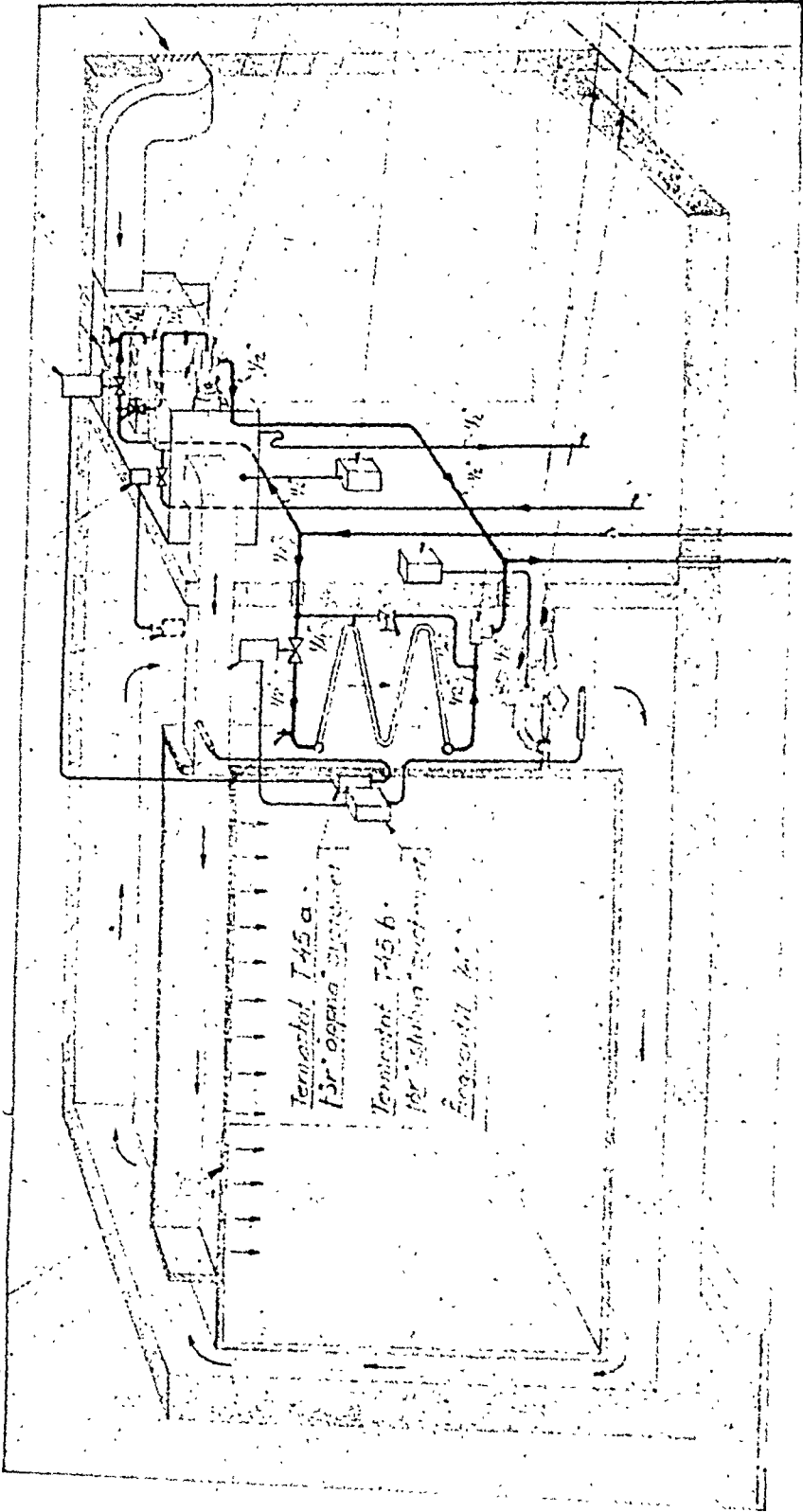


Fig. 1.

sashes — making altogether four window sashes inside one another (see fig. 4). The innermost air space between these sashes communicates with the air space between the double walls, the space next to it with the air space under the window, where the ordinary heating radiator still functions. The outer sashes are coupled together as before. The space between the old door of the room and that built in the new wall has been arranged as an air lock, where

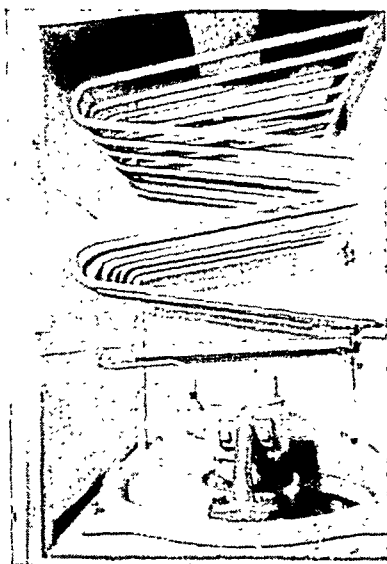


Fig. 2.

the staff and visitors stop a while to regulate the temperature of their clothes before entering the tropical chamber. Alongside this air lock is a large air drum that communicates with the air jacket within the double walls, floor and ceiling. This drum is fitted with a large horizontal fan (see Fig. 2) that sets the air in motion, the latter then passing first of all a large heating coil, which heats it to the required temperature.

The air for the tropical room itself is drawn through a large drum from the open air. After passing through an air-conditioning plant in the corridor outside the room, it is blown into the tropical room through a central grating in the inner ceiling, surplus air being afterwards pressed out of the room through exhaust conduits that run round the edge of the inner ceiling (see Fig. 3).

If the regulating thermostats (see fig. 4) — one for the air

jacket in the walls, ceiling and floor, the other for the air of the room — are set at the same temperature, which has always been done here during the patients' stay in the tropical room, there

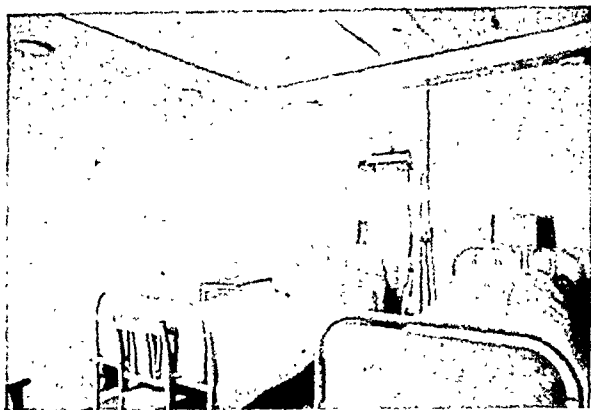


Fig. 3.

will be at most only insignificant temperature-differences amounting to same tenths of a degree between the air in the walls floor, ceiling, inner windows, and room. As a result there will be an absence of convection currents and very little air movement

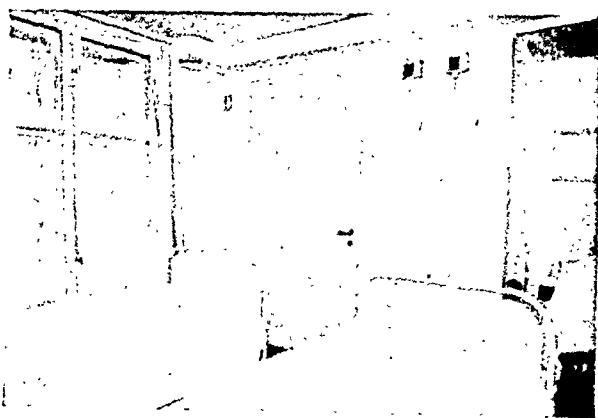


Fig. 4.

in the lower parts of the tropical room. So-called draughts will be entirely excluded.

The relative air humidity in the tropical room is adjusted by means of a psychrostat.

After a period of testing to see what temperature and relative air humidity were felt by the patients to be agreeable and less

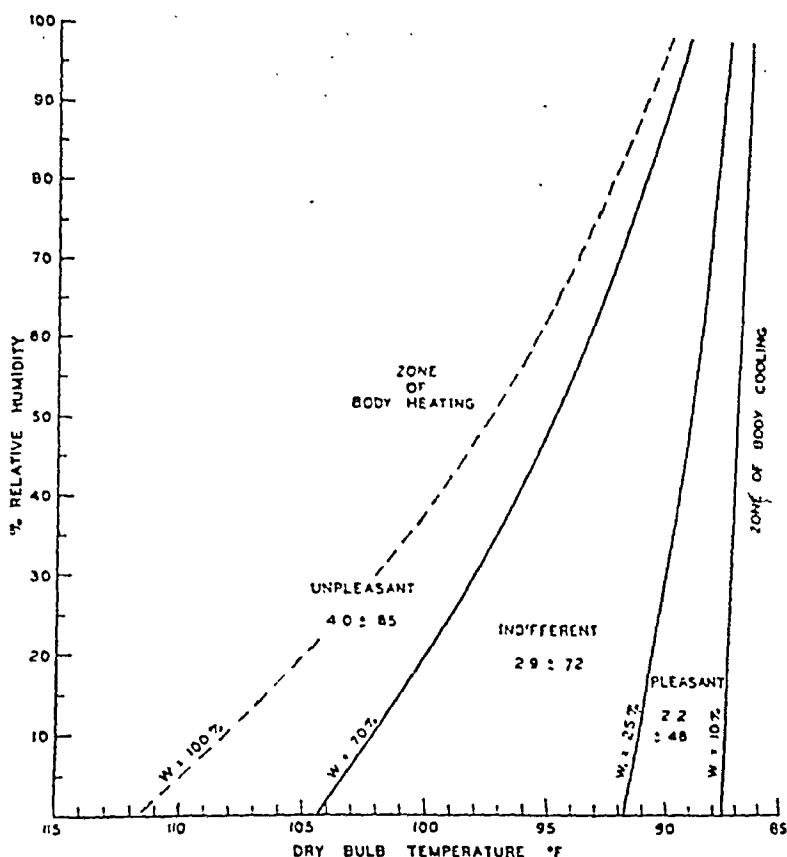


Fig. 5. Contour chart indicating limiting wetted areas associated with certain sensations of pleasantness (in the zone of evaporative regulation) in relation to air temperature and relative humidity. Actual mean comfort votes recorded for each region are indicated. (After Gagge, Herrington & Winslow).

agreeable we came to the same result as Gagge, Herrington and Winslow, (Fig. 5), viz. that chiefly 32°C was regarded by the patients as very agreeable and that under this temperature they were not troubled by any perspiration worth mentioning if the relative humidity of the air was kept below 50 %. With increased humidity the patients preferred a somewhat lower temperature.

For the following tests we accordingly stopped at a temperature of 32°C and a relative humidity of 35–40 %, corresponding to a rather hot but relatively dry tropical climate, a little drier and hotter than that of Porto Rico.

The room could accomodate two patients at the same time, and while there these were kept mainly in bed and were only allowed to be up a short while daily for toilet purposes and exer-

cise. They were allowed — if they could — to attend to their toilets themselves but were strictly told not to use cold water for the purpose. They had no other clothes than a couple of short knitted cotton breeches and the women a short cotton gown; sometimes they had over them a thin cotton sheet. For amusement they had reading and radio. No physical work was allowed. A slight perspiration occurred at times, especially after the patients woke out of a while's deep sleep, but the perspiration was never of a troublesome nature. A Philip's ultra-violet radiator was installed in the room, and the patients were given up to 30 minutes daily irradiation with this. They received the same food as other patients of the clinic — though in comparatively plentiful quantity so that they could choose to some extent. As vitamin preparations, beer-yeast and scorbon were given and, as in the case of the rest, salicylic preparations — with some exceptions, who received amidopyrine or no such preparation. Joint function was supervised and medical movement therapy was given to the extent such was necessary. The treatment in these respects was not allowed to deviate to any extent from what the other patients received while they were inmates of the clinic. No hydrotherapy or other extra thermotherapy was however given to the tropical-ward patients. During their stay in this ward they were never allowed to leave it. Visits from without were permitted. Some of the patients, especially the ladies, were troubled a little by this seclusion.

During the first two years (from October, 1941) that the climatic laboratory in the rheumatic department of the Lund Hospital has been in use 15 cases have been treated in it, seven of which under the diagnosis of *Febris rheumatica* and eight under the diagnosis of *Polyarthritidis rheumatica chronica*. The time may therefore be considered ripe to give a first preliminary account of the results.

Of these 15 patients, 13 were clinically typical but markedly malignant cases of the two types of the disease — the *Febris-rheumatica* cases resembled in much the previously mentioned Coburnian cases, with several organs attacked, transferred from New York to Porto Rico. One of the rheumatic-fever cases (No. 431/42) was complicated by a subsequently arising sepsis, under

which picture it later terminated in death. One of the chronic polyarthritis cases (No. 184/42) was a Still's disease with its specific clinical picture. Although this patient was certainly favourably influenced during his first stay in the tropical room, he soon fell ill again and was then no longer influenced in the same favourable manner, at any rate during his second stay in the tropical ward. All the cases were such as had not hitherto been improved under the customary therapy or had shown severe recurrences.

During their stay in the climatic laboratory all of these patients had been subjectively well, had slept well, and not been nervously irritated by the heat. An exception was a woman of 46 years with malignant chronic polyarthritis (No. 140/42), who at the same time suffered from mental depression and had been treated for this earlier. Her psychical condition became temporarily worse during her stay in the tropical ward, so that this had to be cut short. Nor were we able to obtain any considerable favourable effect of a permanent nature on the rheumatic process in this case.

No change in *body temperature level and in diurnal variations* has been observed in these patients as compared with other patients of the department; nor in the *pulse curves*. The differences noticed appeared to be fully accounted for by the effects of the rheumatic infection, cardiac affections occurring in most of the cases:

No effect on the *rate of breathing* could be observed either.

Nor could any influence be traced as regards the *morphology of the blood* — the haemoglobin as well as the number and type of the *red and white blood cells* showed no other changes than could be put in association with the rheumatic infection.

An interesting observation was made, however, almost immediately after the tropical ward had been taken into use. *At both venipuncture of the cubical vein and finger-tip puncture we could observe a brighter red colour of the blood*, that is to say, the same observation as Robert Mayer made on his sailors after arriving at the port of Surabaja 100 years ago and one that has subsequently been a constant observation in the tropics. We also succeeded in elucidating the cause of this (see Acta Med. Scand., 114, 470, 1943). It lay in the fact that the *relative oxygen saturation of this venous blood had increased*. On the other hand, the haemoglobin was unchanged. Mayer's 100 years old hypothesis has thus been corroborated.

As regards the *blood chemistry*, the first patients were regularly examined in respect of their *serum-Cl*, *serum-Ca*, and *serum-K*. No displacements in these values could be detected. No doubt this is connected with the fact that no very powerful perspiration occurred in these patients. Of course, an increased perspiratio insensibilis must have occurred under the influence of the raised outer temperature. It does not seem, however, to have been sufficient to cause variations in these salt values of a magnitude that could be detected by our routine methods of clinical examination.

Those changes which Borchardt, de Langen and Schut, Sundström *et al.* have found in these values among individuals in the tropics would also seem, as most of these authors consider, to be associated with an increased perspiration.

The *total cholesterol values* were displaced in some of these patients, but as these values are also displaced in the chronic forms of rheumatic polyarthritis, and as no exact investigations in this respect have as yet been conducted, this problem will be a matter for closer treatment in the future. The same applies to the *blood sugar* (Cf. Andrews and Muether).

Metabolism and calorie consumption. All food consumed by these patients before, during and after their stay in the tropical ward was weighed and measured, and the caloric value was then calculated by Klarin's tables. The values obtained calculated as the mean values of three days were plotted into diagrams. Fig. 6—7—8—9—10.

In some of the cases considerable fluctuations in food intake were found. However, if account is taken of all the accompanying changes in weight, and increased weight is calculated as stored fat, it will be found that in general the basal metabolism did not exhibit so great variations.

Certainly there may be striking exceptions, as in case No. 184/42, the juvenile case of chronic polyarthritis of the Still's disease type. Here it is observed that on entering the tropical room the patient, with a body weight of about 28 kg, had a total daily consumption of about 800 Calories. This is quite too low a consumption as against what we consider to be normal for this age, 40 Calories per kg, i. e. about 1,100 Calories. It will also be seen that the patient's weight fell during the nearest time. The values are however explained by his extremely poor general condition and the

Nr. 368/41 O.V.B. D. b. 1912

Diagnose: Febris Rheumatica.

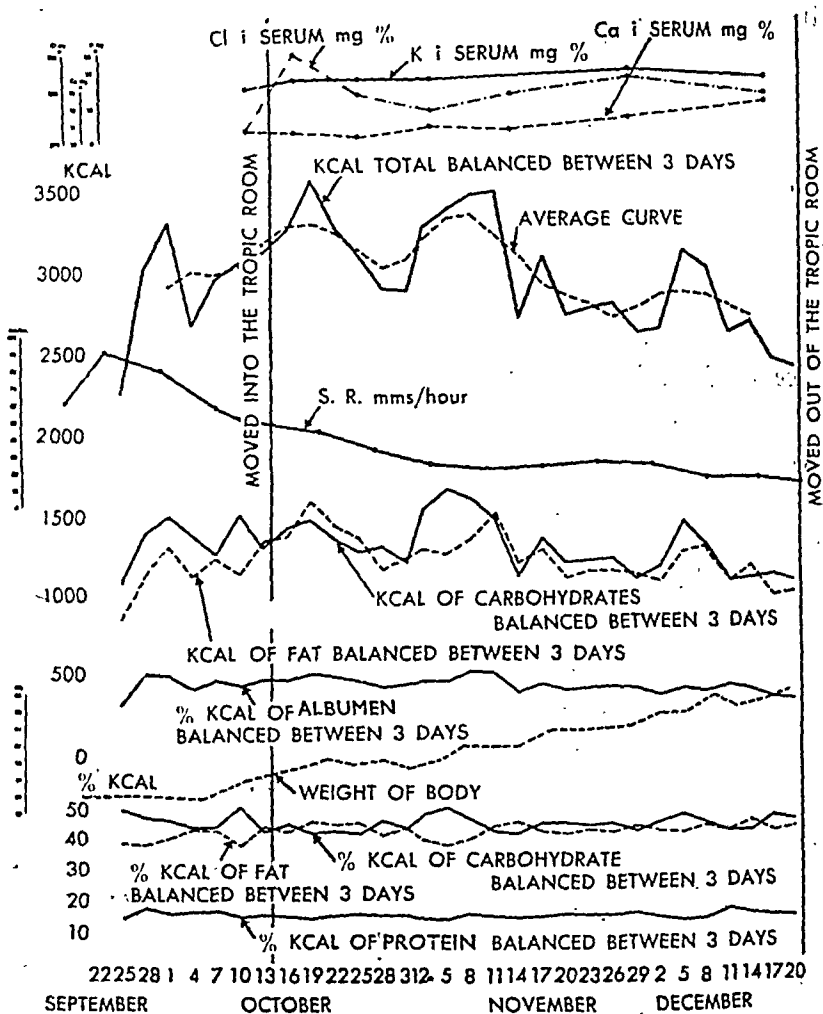


Fig. 6.

intense infection present at this time. The patient then began to improve slowly and there was a parallel increase in food consumption. It is interesting to observe that, although the rather striking clinical improvement did not set in until after wellnigh three months, the patient's appetite began to increase much earlier. The coming clinical improvement can be foreseen on this diagram. The body weight of the patient was lowest in June-July, about 24 kg, and

Nr. 454/41 B I O. C b. 1927
Diagnose: Febris rheumatica

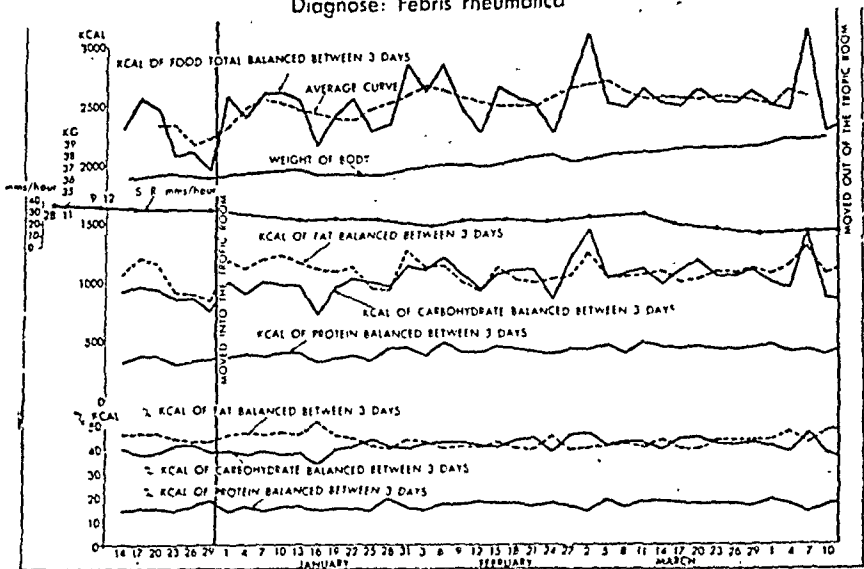


Fig. 7.

Nr. 366/41 S. E. N. b. 1920
Diagnose: Polyarthrititis rheumatica chronica

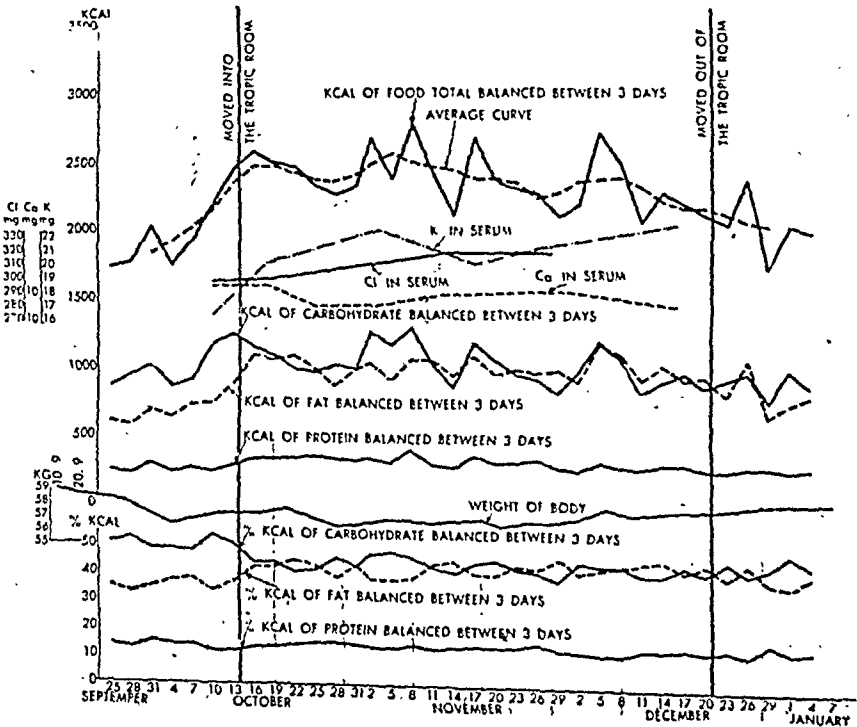


Fig. 8.

Nr. 96/43 M. E. K. P. b. 1903
 Diagnose: Polyarthrititis rheumatica chronica

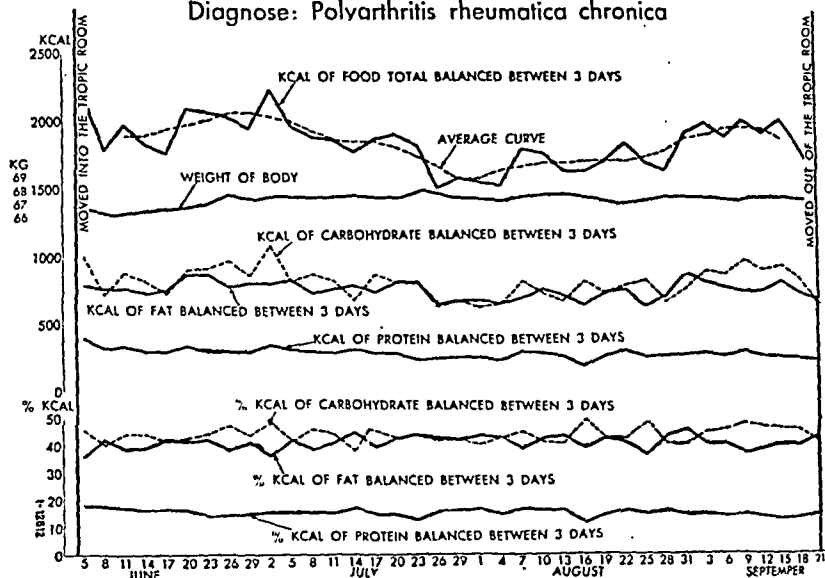


Fig. 9.

Nr. 350/42 H. A. H. b. 1893
 Diagnose: Polyarthrititis rheumatica chronica

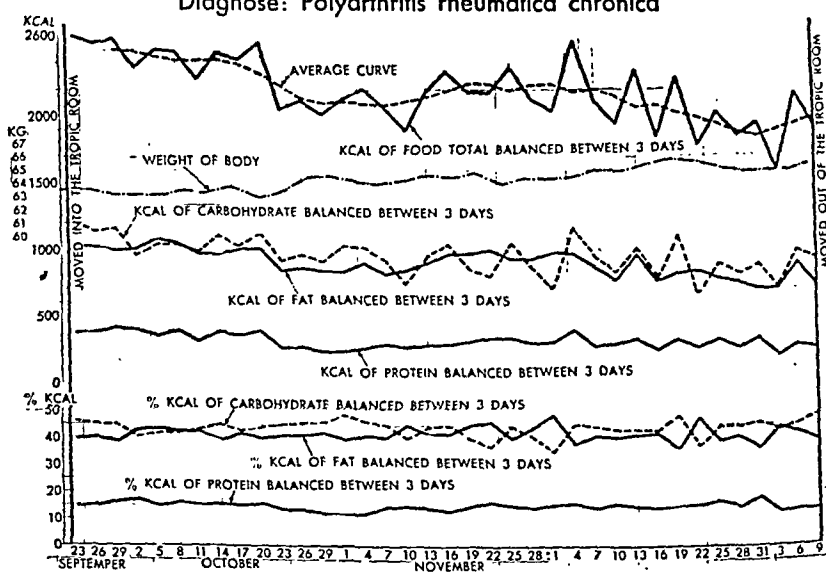


Fig. 10.

thereupon it began to increase. He still had fever. By the beginning of July his food intake had reached about 1,400 Calories, which was more normal for his weight and age, his weight becoming stabilized. Then his intake of food slowly mounted to about 2,500 Calories, his weight increasing in the same proportion. By the end of January, 1943, he weighed 39.5 kg.

These great variations in food intake can however be explained partly by the patient's poor general condition and febrile state during the first part of the time and partly by his increase in weight during the latter part of the time. A diminution in food requirements produced by the tropical climate is impossible to read. When the weight remained the same and the morbid process had calmed down in the month of July, the intake of food was at the normal value of about 1,400 Calories for his weight, age and decombiture.

If the other diagrams showing food intake were examined in the same way with due reference to the infection and weight-curves of the patients, the same result was reached. On the whole, no other conclusion could be drawn from the investigations carried out here than that there had been no displacement in the total consumption of calories during the time the patients had stayed in the tropical ward.

In a case or two — e. g. patient No. 368/41 — a slight increase in the consumption of calories was noticed during the weeks immediately following admission to the tropical ward. This observation agrees with Knipping's observation that sometimes a slight increase of this kind is obtained in the basal metabolism at the beginning of «a tropical stay». This slight initial rise, however, passed off after a few weeks in those cases in which it occurred here.

If a depression of the basal metabolism had occurred, i. e. if the second chemical regulation of temperature had entered into function, it would have been expected to take place chiefly during the latter part of the stay in the tropical room, when a certain amount of acclimatization ought to have been reached. As a matter of fact, in four of the cases — patients Nris 368/41, 84/43, 350/42, 310/42 — a slight depression of the total consumption of calories could be read during this latter part. It must not be forgotten, however, that in these cases the stay in the tropical room had brought about an improvement in the rheumatic process, with

consequent changes in the body. During the state of infection, especially the state of fever, the basal metabolism was mostly increased. If this state was improved, return to normal conditions took place. An explanation on these lines cannot be entirely excluded here, even though the temperature was on the whole normal in these cases. It is possible, though, that a slight depression — up to 10 % — in the basal metabolism had really occurred in these instances during the latter part of the stay in the tropical ward. A greater depression can be excluded with certainty in these cases as well.

These results agree with what has been found in the tropics. For instance, Glogner, in the course of a journey in the tropics, during which he carefully measured his own consumption of calories, could not detect any alterations in it. Eijkman as well as Caspari and Schilling also came to the same result, as did G. and O. Fischer and Borchardt later. On the other hand, Ozario de Almeida considers he found a lowering of the basal metabolism of about 15 % in the tropics of Brazil, Knipping, using more refined methods, arrived at about the same result (10—20 %), as did also Sundström. Finally, Young thinks he has found an increase, especially during the very hottest rain period.

Loewy considers that an explanation of these somewhat divergent results may lie in the fact that the processes of acclimatization and adaptation play a large part and exercise an influence when white people come to the tropics. This acclimatization, which during the first part of the time evokes an increased basal metabolism similar to the initial increase obtained here in some of the patients during their first few weeks in the tropical ward, can, Loewy's thinks, explain to a certain extent Knipping's and Sundström's results, which were obtained on whites who had recently come to the tropics. Young's result, on the other hand, is explained by a hyperthermia that had arisen during the hottest rain period.

The non-appearance, on the whole, of any change in the consumption of calories and in the basal metabolism under normal tropical conditions — the same result as obtained here in the artificial tropical climate — also agrees with the results to which Wezler and Thauer recently came in some physiological experiments (see fig. 12).

These authors conducted experiments in a climatic chamber of similar construction to that used here, but in which the subjects were healthy individuals who were kept only a short while in the chamber under a temperature that was changed within wide limits. The experiments yielded the result that under physiological conditions, if oxygen consumption and metabolism are taken into account, three temperature zones can be distinguished: (1) a zone under 22°C with an oxygen consumption and metabolism that rise the higher the colder the outer temperature is, (2) an indifferent zone between 22°C and 36°C with relatively low oxygen consumption and metabolism, which falls somewhat with rising temperature — individually rather different —, (3) a zone above 36°C , again with increased oxygen consumption and metabolism, rising the higher the hotter the temperature.

Wezler and Thauer's investigations thus agree with our results. With most individuals we have not any real diminution of the processes of burning in the organism at the degrees common in the tropics. The so-called second chemical regulation of temperature — about which so much conflict has waged — does not at any rate function in the majority of individuals under these conditions. But Wezler and Thauer's investigations also supply an explanation for the somewhat divergent results at which a number of observers have arrived and which have also appeared in a couple of cases examined here, these authors having shown that certain individuals are rather labile as regards their heat-regulating mechanism. In these a rather pronounced metabolic minimum is found between 30°C and 36°C .

It is self-evident that a displacement in the physical regulation of temperature occurs at these high temperatures of the air surrounding the body. At a common room temperature of 18°C . only about 12 % of the amount of heat produced in the human body is given off through evaporation of water from the body surface and the lungs, while about 8 % is lost by warming the food, and about 80 % by radiation, conduction and convection. At 29°C . with a not overhigh relative air humidity, however, the amount of heat lost through evaporation is, according to Knipping, about 70 % of the total, and at a temperature of $32\text{--}33^{\circ}\text{C}$. this rises, according to Borchardt, to 85 %. Gröber's figures agree with these (see Fig. 11).

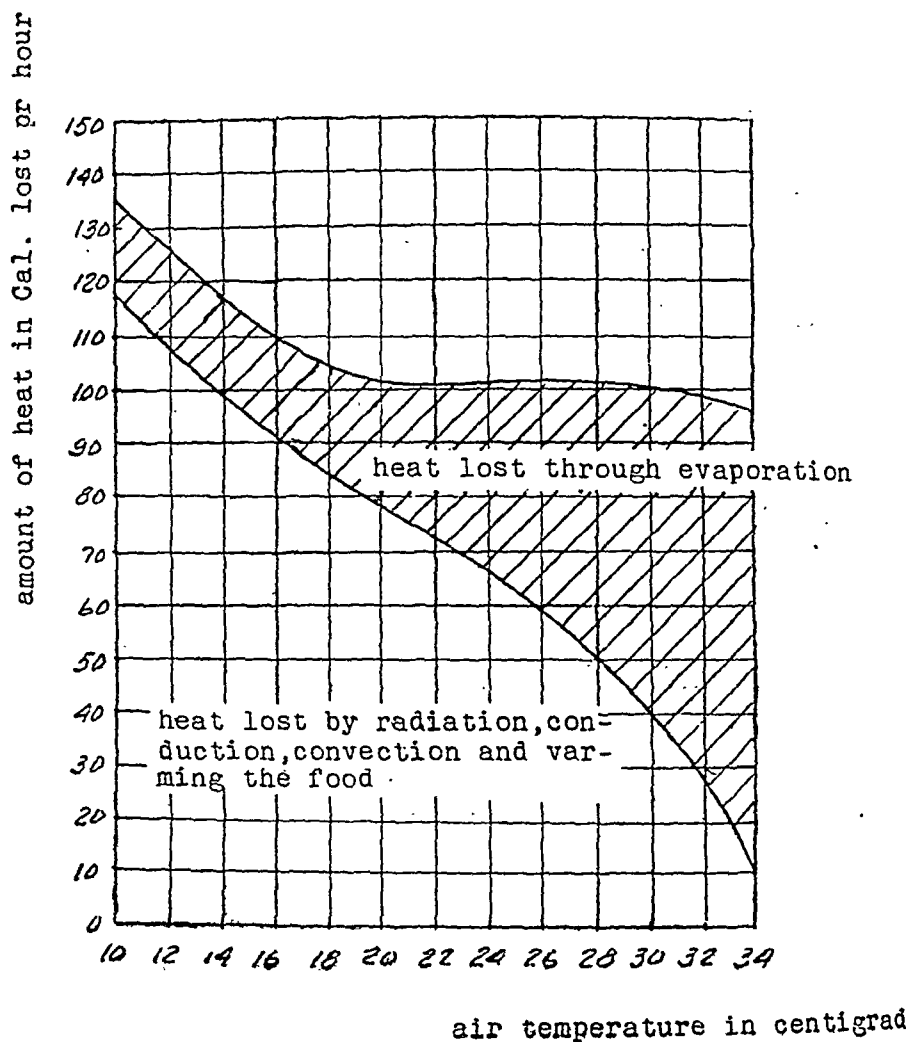


Fig. 11. Amount of heat produced in the human body given off by very light clothes and stagnant air. (After Gröber).

An intervention of the so-called second chemical regulation of temperature might, therefore, be expected. That this occurs only in individuals of a rather labile constitution is rather surprising, but is in full agreement with, for instance, the results to which Edström came in regard to another climatic factor, viz. the electric air ions. Here, too, there is very great individual variation and many entirely insusceptible individuals.

Of interest are the investigations of Hardy, Mildhorst and du Bois, according to which females show this so-called second chemical regulation of temperature more often and more markedly

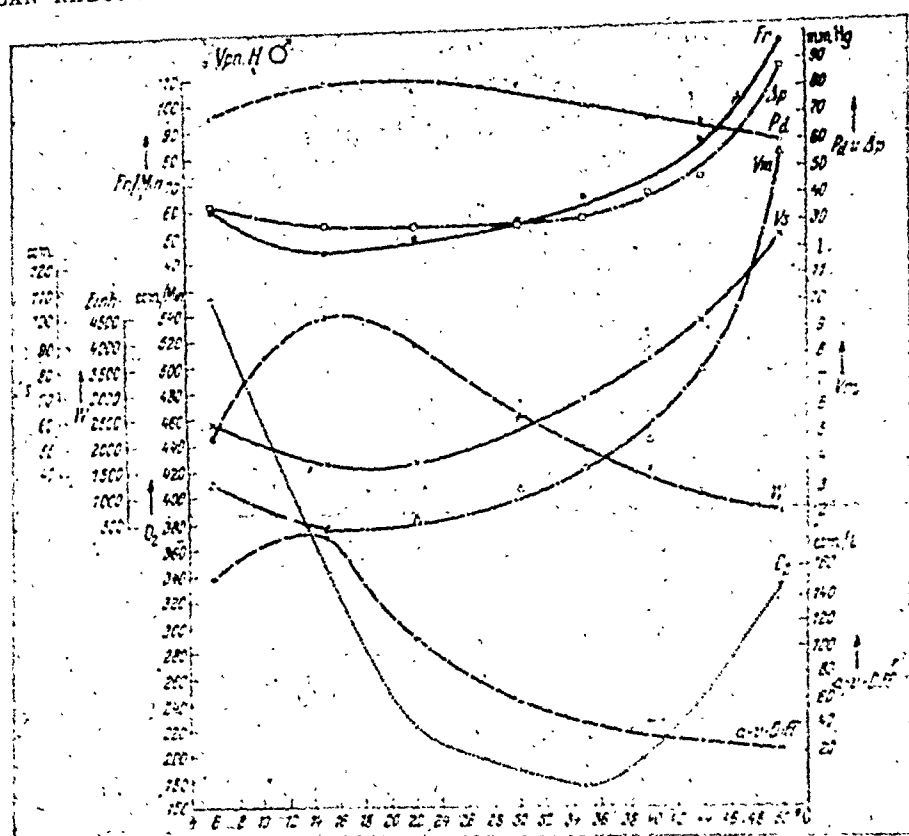


Fig. 12. The experiments of Wezler and Thauer in a climatic chamber. The subjects were healthy individuals who were kept only a short while in the chamber under a temperature that was changed within wide limits.

P_d — diastolic blood pressure; Fr — pulse rate; Δp — the amplitude of blood pressure; V_m — the minute volume of the heart; V_s — the cardiac output; W — the active peripheral resistance to blood flow; O_2 — oxygen consumption pr minute; $a-v$ Diff. — the arterio-venous difference (the utilization). After Thauer & Wezler.

than males. These authors found a depression of the basal metabolism more often and constantly in young women than in young men, if the temperature of the outer air was raised from 22°C . to 35°C . This difference between the sexes was not confirmed by Wezler and Thauer in their small material, but here, too, there ought to be a wide individual variation.

At temperatures above 36°C ., finally, there sets in a hyperthermia of the body with a consequent increased oxygen consumption and metabolism (Wezler and Thauer) in the same way as in fever (du Bois).

If attention is then directed to the percentages in which protein, fat and carbohydrates enter into the food eaten by the patients

during their treatment in the tropical ward, it will be found that especially the protein percentage is constant before and after this treatment. It keeps roughly round about 15 %.

There are however certain deviations. To explain their import, however, the value of the different foodstuffs for the body must first be considered. Carbohydrates and fats are almost exclusively sources of energy. The principal function of the proteins in the body is to provide material for the building up of new tissues and for the metabolism going on in the cells. For the last-mentioned purpose we require roughly ten different amino-acids. The value for the body of a protein thus lies in the quantity and kind of the amino-acids that enter into it. The animal proteins are from this point of view of fuller value than the vegetable. Work or rest influences the energy requirements but not the protein to any noteworthy extent. To be well nourished, an adult requires about one gram of protein per kg daily — it is usual to calculate with about 80 gms per individual of 70 kg. The protein minimum, however, is lower, viz. about 45 gms, though at least one-half of this is animal protein. During growth and during fever as well as certain other morbid conditions more protein is required.

The patients treated in the tropical ward, as already stated, were resting all the time, mostly lying in bed both during their stay in the ward and before, after as well as during the measuring of the foodstuffs. Under these conditions the protein requirement derived from the food ought to have been constant in percentage, unless the climatic factor had exercised an influence. Evidently there was no such influence. The deviations obtained, as in case No. 184/42, are entirely accounted for by the infectious state at the time or by overfattening. This patient had, e. g. during the second month of his stay in the tropical ward, a relatively high protein percentage, averaging during this month 18.4 %. But at the same time the total absorption of calories was very low, about 800 Calories, and he did not even get his normal protein minimum for a growing individual, which was in this case further elevated on account of the fever then present. Towards the end of his treatment his absorption of calories rose to 2,500 Calories, but his protein percentage dropped to 13 %. Now he had come into the fattening stage and he got more than his normal protein requirement.

Even though the fat consumption and the carbohydrate consumption did not show the same constancy in these patients as the consumption of protein they exhibited no considerable variations. In a couple of cases, though, some slight decrease in fat consumption and an increase in carbohydrate consumption were observable.

This relatively constant proportion between the different groups of foodstuffs was a very interesting finding. Certainly these patients had not had entirely self-selected diets, their choice having been limited by the hospital food offered them. However, this was intentionally abundant, and, as they were able to get extra food to the extent they desired, there was some degree of self-selection. Hence Jones's results as to the constancy with which the individuals maintain these proportions between the different groups of foodstuffs in selfselected diets have been confirmed.

As regards the consumption of protein, however, the financial standard of the individuals plays a certain role. When the standard is high, there is likely to be a higher protein consumption (Widmark). The finding of a low protein consumption in a number of investigations on natives of the tropics — Mary Rose, for instance, found that in the Bengali it was below the protein minimum and at 9 % of the food calorie value — is therefore probably connected more with social and economical standards than with climatological factors. The same may be said of Krogh's values from Greenland. He found that the Eskimos of Greenland consumed a daily average of about 280 gms protein, 135 gms fat, and 54 gms carbohydrate, or 44 % protein in terms of calorie value. It is certainly true that they felt extremely well on this fare, but when communications afforded them the opportunity of getting more carbohydrates they partook of these with delight and at present their carbohydrate consumption has substantially increased. It is therefore hardly justifiable to call their old fare a self-selected diet. Hoffman's find that »the protein, carbohydrate and calory consumption is almost identical among the various peoples», which he partly based on Rubner's studies of the average food consumption of various peoples, Japanese, Italians, Russians, Germans, Frenchmen, Britons, Americans of the U. S. A. and of Central America, i. e. people living both in the tropics and in the temperate zones, must presumably still be considered correct

and is not contradicted by these investigations. The fact that the fat and carbohydrate consumption varies more than the protein consumption on transition from rest to work has already received mention in this paper. Stepp's and Voit's observations in this direction probably rest on this base.

In financially well-situated Europeans in the tropics Eijkman found a somewhat lower protein consumption and protein percentage in the food than in the corresponding group of people in Europe, from where they had come — 14.4 % as against 19.2 % —, but the investigation covered only a few persons and it is not quite certain that their choice of food in the tropics had been entirely free.

Circulatory conditions. Wezler and Thauer's investigations have made it clear that with rising outer temperature and under ordinary physiological conditions there is, contrary to the case with metabolism and oxygen consumption, no indifferent zone in the case of the circulatory conditions. Neither are an oxygen consumption minimum and metabolism minimum of about 30°—36° C. represented by any corresponding minimum loading of the circulatory mechanism. The cardiac output and the minute volume of the heart present their minimum values under a common room temperature of about 18° C., whereas the active peripheral resistance to blood flow and the arterio-venous difference — the utilization — fall with rising temperature over the whole range of temperature from common room temperature to 50° C. (see Fig. 12).

Just as Wezler and Thauer were able to find a certain depression of the *diastolic blood pressure* in their physiological material when the outer temperature had been changed from 20° C. to 32° C., so could the same slight depression also be observed in this material. This depression, which may be said to be a sign of the reduced active peripheral resistance to blood flow, is no doubt, like the abovementioned increase observed in the relative oxygen saturation of the blood in the more superficial arm veins, due in the first place to the fact that the arterio-venous anastomoses in the peripheral parts — chiefly fingers and toes — open up under the heat effect brought into play, in the same way as when the hand is held in hot water.

In the rheumatic states of disease — chiefly the chronic forms of rheumatic polyarthritis — it is fairly certain that these anastomoses as well as the capillaries and the subcapillary arterioles are in a certain degree of spasm. It is principally this spasm which is relaxed in the tropical ward here and replaced by a state of considerable dilatation. The peripheral vasoconstriction in these states of disease, which is a typical clinical feature (Edström: Acta Med. Scand., 103, 90, 1940), and which is a foundation for several of the clinical syndromes so troublesome for the patients, is abolished at this temperature and a good peripheral blood supply takes its place. This can also be read from the relative oxygen saturation values obtained in the blood of the surface veins. In normal room temperature a relative oxygen saturation averaging 51.3 % by volume was obtained (see Acta Med. Scand., 114, 470, 1943), a value that is substantially lower than that obtained under ordinary physiological conditions — about 70 % by volume according to Liljestrand and Lundsgaard. After the patients were placed in the tropical ward with its 32° C. this relative oxygen saturation immediately rose to above 80 % by volume — here averaging 81.5 % by volume. We have seen that no essential change in the metabolism and oxygen consumption in the tissues had taken place. So great a displacement of this consumption that it could be the cause of such a considerable rise can be excluded. The cause must accordingly be an improved peripheral flow.

This is also evident from the state of the *skin temperature*. In the chronic forms of rheumatic polyarthritis the peripheral parts of the body, especially hands and feet, have an exceptionally low skin temperature as a symptom of the impaired peripheral circulation (see Acta Med. Scand., 83, 523, 1934 and 103, 90, 1940). The same observation was also made on the patients examined here. But after transference from common room temperature to the tropical ward, 32° C., this skin temperature rose substantially and more powerfully than the skin temperature of the trunk. In some cases the interesting observation was made that the skin temperature of hands and feet was even higher than that of the trunk. This must imply a very powerful dilatation of the arterio-venous anastomoses. A more detailed account of this will be given later.

The Clinical Status.

All the *febris rheumatica*-cases were extremely malignant, with polyarthritic syndromes and an endomyocarditic syndrome. Only in one case was the latter missing. In one of the cases there was also a pericarditic syndrome, in three of them a nephritic syndrome, in two of them a peritonitic syndrome. In two of the cases there was an encephalitic syndrome and in two a conjunctivitic syndrome.

The case histories tell us, that the clinical status improved almost immediately after the patients had been admitted to the tropical ward, and that after even a couple of weeks all of them had a different and less affected appearance. Especially the cardiac function had changed for the better, the pulse rate had gone down, and in those cases in which dyspnoea and cyanosis were present these symptoms had vanished almost immediately.

The effect of increased outer temperature on cardiac compensation has not been fully elucidated. The above-cited investigations of Wezler and Thauer show that the cardiac output and the minute volume of the heart are slightly increased, whereas the active resistance to the peripheral blood flow and the utilization are reduced. The two first changes render the work of the heart more difficult, the latter render it easier. How does the summation effect present itself? Mills, on the basis of a particular hospital clientele, has studied how the cases marked by heart incompensation not due to a violent attack of infection are distributed over the different months with different mean temperatures (see Fig. 13). He has found a strictly inverted relation between the curve for the mean temperature and that for the average number of attacks. The higher the temperature, the fewer cases of heart incompensation. But the mean temperature in Cincinnati, where the investigation in question was carried out, does not exceed 80° F. (27° C.). The experience gained in Lund suggests that the same rule also applies to somewhat higher temperatures, that in any case the temperature of 32° C. in use here with a comparatively low relative air humidity of 35—40 %, under strictly resting conditions, has a beneficial influence on the functional state of the heart, which must quite naturally be of considerably importance for rheumatic-fever cases in which the cardiac affection co-dominates.

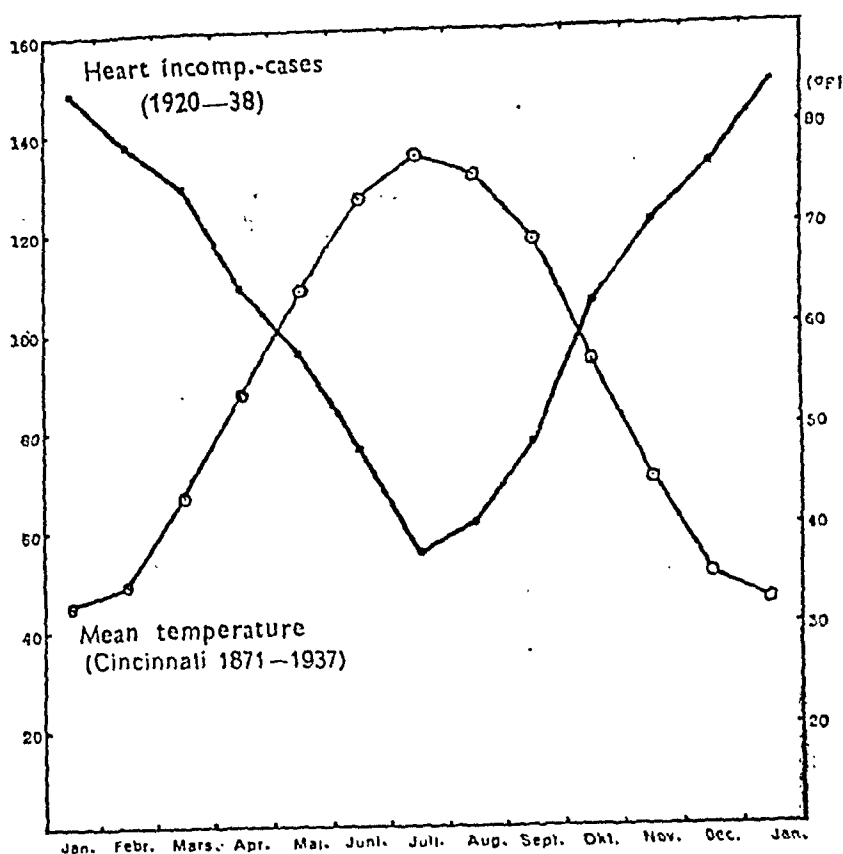


Fig. 13. Number of heart incompensation cases among the patients in the General Hospital in Cincinnati. The average number of attacks not due to a violent attack of infection in the years 1920—38 show a strictly inverted relation to the curve for the mean temperature of Cincinnati in the years 1871—1937. After MILLS.

tes with the articular. This observation is of importance, as there seems to be a fairly widespread view that a relatively high room temperature is not so advantageous for cases with a poorly functioning heart.

A nephritic syndrome was found in three of the cases. Two of these were relatively mild with good concentration power to thirst tests, normal urea-clearance, normal blood pressure, only periodic traces of albuminuria, and a few million red blood cells in the urine sediment on quantitative tests. The syndrome in these cases subsided rapidly.

The third case (No. 431/42) was complicated however. This case, too, showed a striking improvement in the general condition at the



Fig. 14.

beginning of the tropical treatment, this improvement continuing during the first few months, but the renal symptoms were rather persistent, with a relatively high albuminuria (about one per mille), numerous red blood cells in the urine sediment, although the blood pressure, non-protein nitrogen, indikan, xantoprotein, and thirst test were normal. The blood sedimentation rate also receded in this case as in all the others, falling almost to normal values, and the process appeared to be in course of healing, even though Erythema-annulare efflorescences (Fig. 14) and transient articular symptoms appeared off and on.

Then an acute tonsillitis arose. Culture from the throat flora showed a pure strain of streptococcus viridans, not hemolytic in flat culture. Almost immediately there followed an acute deterioration in the condition, chiefly in kidney function. Then a somewhat elevated blood pressure and increased nonprotein nitrogen were registered. The patient, who had previously had an encephalitic syndrome, again began to exhibit marked hypotonia of the muscles — jerkiness of the arms reminiscent of

chorea minor and nervous restlessness. He was irritated by the seclusion, and, as there was no change in his condition after some weeks, his stay in the tropical room was broken off and he was transferred to a sick-ward with ordinary room temperature. After being taken out he did not become worse, neither did he get any better. Gradually, however, his state deteriorated, and some months later he died of uraemia. The renal function, however, was not bad until the last few weeks; nor was it bad during his stay in the tropical ward.

Besides this case there was an encephalitic syndrome in another one, in both instances during the poor condition when the patients were placed in the tropical ward. The symptoms consisted of marked muscular hypotonia, jerky movements of the choreatic type without being definitely choreatic, and a certain amount of mental confusion for some days. In one of the cases there was an acute psychotic state for some days with illusions and hallucinations. No particularly favourable or unfavourable effect could be observed on this syndrome by the transference to the tropical room, the course of the syndrome following the intensity of the total morbid picture in each case.

The polyarthritic syndrome was obviously improved in all cases immediately after the patients were placed in the tropical ward. In this respect the most conspicuous clinical change occurred in the same way as in the cases of chronic rheumatic polyarthritis.

The total clinical improvement in all these cases after they had been placed in the tropical chamber was remarkable. It is to be noted, however, that ordinarily a good improvement also occurs in most of the rheumatic-fever cases after admission to a hospital with common room temperature and with the customary medical therapy. The cases treated here, however, were selected bad ones that had not improved earlier and that, as previously mentioned, much resembled Coburn's cases. With the exception of the case just described that terminated in death from uraemia, all of these cases were discharged later free from symptoms, though certainly in a couple of cases with remaining scar-formations in the form of vitium of the heart. They have so far remained symptomless, and the patients have taken up full-time work or attended school.

The throat flora of these patients was regularly examined almost every week by means of cultures before, during and after their

Table 1.

Patients with febris rheumatica treated in the tropical ward of the Rheumatic-Department of the Lund Hospital during the period Oct. 1941—Oct. 1943.

Patient	Age years	Days in tropical ward	Result		Flat hemolytic β -streptococci in throat	
			Imme- diate	Final	On entering	On leaving
O. V. B. D.	28	70	good	well	+	—
B. I. O. C.	14	106	good	well	+	—
A. D.	13	106	good	well	+	—
S. B. S.	17	126	bad	dead	—	—
T. B. B.	14	79	good	well	+	—
P. H. P.	17	43	good	well ¹	+	—
M. O. L. Å.	27	53	good	well ¹	—	—

¹ These cases have only been under observation two months since the treatment.

stay in the tropical ward. In five of the cases β -streptococci hemolytic in flat culture were found in fairly copious amounts at the time the patients entered the tropical ward, in no case on their leaving it. Nor did these bacteria recur in the flora afterwards in any of the cases, but the period of observation was short — only some weeks, and therefore it is not yet possible to pronounce any opinion on this tendency in the long run.

All the chronic rheumatic polyarthritides cases were also selected malignant ones, with a rather severe polyarthritic syndrome. Four of them also had a peritendinitic syndrome, and one a neuritic syndrome. In two of them there was an endomyocarditic syndrome. The pericardium was also involved in one of these, a juvenile case clinically of the Still's disease type, with considerable enlargement of the spleen. There was an achylia in one of the cases, and a keratoconjunctivitis sicca in another. In one of the cases a subcutaneous nodulus was extirpated that histologically presented a typical rheumatic-tissue picture.

As will be found from the case-histories, the clinical condition improved in all these cases, as in the acute series, almost

immediately after the patients had been placed in the tropical ward. Cardiac affection, when present, was influenced in the same way as was previously described for the rheumatic-fever cases: improved heart function was established. An extremely striking feature was that the blue-livid coloration of skin on hands and feet vanished in those cases in which it had been present. Both hands and feet assumed a rosy tint, warm with — as already mentioned — a high skin temperature.

The most powerful effect of the tropical treatment, however, was in most cases on the articular affection. A fairly rapid remission was especially noticeable in the peri-articular oedema of the joints attacked as well as in the capsular swelling and any intra-articular exudate that was present. That was a rule without exception — the rapidity of the process might certainly be variable, but in all of these cases, even in those in which the final result was not so good, the objective status of the attacked joints improved very considerably in this respect during the stay in the tropical ward, while the subjective symptoms in the form of tenderness on movement and on pressure in these less successful cases were not influenced so much. A characteristic feature was that in a couple of cases all therapeutic possibilities had been previously tried in order to ameliorate the considerable knee exudates, repeated knee punctures, X-ray therapy, short-wave and other thermotherapy, etc. without the least effect, the exudates having persisted for several months up to a couple of years. After a couple of weeks in the tropical room these exudates vanished without any special local measures.

The peritendinitic exudates in the sheaths and bursae, although distinctly influenced, were effected less and more slowly than the corresponding processes in the joints themselves. The achyilia was not affected, but the case in question was affected as a whole relatively little and belonged to those having a bad final result.

As indicated in Table 2, the immediate result in seven of these eight cases was good. Tropical treatment of the eighth case had to be broken off on account of the presence of a *depressio mentis*. In four of these cases the final result was also good, and all of these four — i. e. one-half of these chronic polyarthritis cases — are now at full-time everyday work. In two of the others the rheumatic process started again after only a few weeks. No permanent effect

Table 2,

Patients with polyarthrititis rheumatica chronica treated in the tropical ward of the Rheumatic Department of the Lund Hospital during the period Oct. 1941—Oct. 1943.

Patients	Age years	Days in tropical ward	Result		Flat hemolytic β -streptococci in throat	
			Imme- diate	Final	On entering	On leaving
S., E. N.	21	70	good	good	—	—
K. G. G. J.	11	158	good	—	—	—
do.	12	114	bad	bad	—	—
H. H.	46	49	bad	bad	+	—
M. E. K. P.	39	111	good	bad	+	—
H. A. H.	49	111	good	bad	+	—
R. S. L. J.	22	75	good	good	+	+
H. A. B.	42	64	good	good	+	—
I. D. B.	18	36	good	good	+	+

was obtained in these two cases, both being about as bad as before a few months after removal from the tropical ward.

The seventh of the cases — that with Still's disease — was interesting. On being placed in the tropical ward the patient was in an extremely miserable condition. (See Fig. 15). He had been in the hospital for more than one year without any real improvement whatever, although his condition had shown variations in malignancy. He exhibited periarticular oedema and capsular swellings on most of the joints of the extremities, could not grasp with his fingers, and could not stand without help. He had high-grade muscular atrophy, heart affection, and a considerably enlarged spleen. During his first few months in the tropical ward he was relatively little influenced. His calorie consumption and assimilation of food during this time have already been mentioned. After he had been three months in the ward, however, a remarkable change took place. He had a different appearance, the arthritis subsided relatively quickly, as did also the cardiac affection, he ceased getting leaner and again began to gain weight. The erythrocyte sedimentation rate, which had been very high, dropped to almost normal value. After his removal from the tropical ward this improvement continued and he was discharged from the hos-



Fig. 15.



Fig. 16.

pital, the first time, free of symptoms save for a still enlarged spleen and a faint murmur over the heart. His condition before (Fig. 15) and after (Fig. 16) the treatment can be seen from the illustrations submitted earlier.

After some months' stay at home, however, he had a relapse of an exceedingly malignant character and was re-admitted to the hospital in a highly febrile state, with violent arthritis and peri-articular oedema of most of the joints of the extremities, recurrence of his cardiac affection, considerably enlarged spleen again, and generally effected. This time, too, tropical ward treatment was tried. The result was not so good, however, the boy this time being very impatient and ill at ease in his seclusion. As he did not show any real tendencies to improvement after nearly four months treatment, this was broken off. He is still in a poor condition.

Deducting the eighth patient, whose treatment had to be broken off, the final result will thus be four cases free from symptoms and fully capable of work, three unimproved on the whole.

The throat flora. The throat flora of these cases as well was examined practically every week by culture before, during and after treatment in the tropical ward. In six of the cases there were β -streptococci hemolytic in flat culture in fairly abundant quantity when the patients were placed in the tropical ward. During the patients' stay there, however, these cocci disappeared more or less rapidly and, when the patients were discharged, were found in only two cases, though not in great numbers in either of them. In one of these, however, the patient's stay in the tropical ward had been as short as 36 days. One of the cases, which during the whole treatment had never shown any hemolytic β -streptococci, was the above-mentioned case of Still's disease. It exhibited the whole time streptococcus vididans, non-hemolytic, in practically pure culture.

These experiments therefore, would seem to justify the conclusion that *the artificial tropical climate brought about here seems to have the same effect on the throat flora in regard to hemolytic streptococci as the natural, viz. they disappear in the great majority of cases*

The hemolytic streptococcus seems not to thrive in the tropical climate. Hemolytic-streptococcal affections are, as a matter of fact, relatively rare in the tropics. Erysipelas, streptococcal tonsillitis, streptococcal otitis, streptococcal pneumonia, scarlatina, etc. admittedly occur in a case here and there, but are rare everywhere in tropical climes with their otherwise numerous infections.

In this, then, the explanation of the fact that neither does the rheumatic infection thrive there? The question whether rheumatic infection is a hemolytic-streptococcal infection has long been on the tapis. Since a long time ago numerous voices have been raised, especially in the Anglo-Saxon countries, to the effect that this is the case. The chief obstacle that has stood in the way of a full elucidation of the question has been the fact that owing to the allergic nature of the rheumatic process great difficulties have been encountered in direct attempts to detect the causative antigen (see Acta Med. Scand., 103, 90, 1940). However, since this has been realized and research has been directed instead to ascertaining the extent to which antibodies are present, the question has come considerably nearer solution. Now that Coburn, Tillett, Perry, Hill, Bunim and McEwen as well as several other workers in the U. S. A. have shown that at any rate in rheumatic fever antibodies to hemolytic streptococci are detectable in as high a percentage (between 90 and 100) as in undoubted streptococcal affections, and as the same results have been obtained in our northern countries from corresponding researches by Waaler in Oslo, Winblad in Lund, Lou and Kalbak in Copenhagen, and Packalén in Helsingfors, I have to acknowledge, at least as my own personal view, that the question of the etiology of rheumatic fever seems to be solved. *Rheumatic fever seems to be a hemolytic streptococcal infection.* This does not, of course, exclude the possible occurrence of a symbiosis with a subvisible virus or suchlike. The pathogenesis and the clinical picture are then essentially explained by the secondary reaction of the body, the allergic mode of reaction, in a way that has been clarified especially by Klinge.

Obviously, there is another etiology in solitary cases of what, without rigid bacteriological analyses, we now call rheumatic fever, especially some cases coming in attendance on infections of the bileducts, digestive canal, and urogenital sphere. Strictly

speaking, however, these cases should not be called by this name, but should be labelled as allergic reactions attending these other infections. Just as the concept pulmonary tuberculosis was to some extent narrowed by the exclusion of a number of border-cases when this disease was explained as being due to the tubercle bacillus, in the same way the concept rheumatic infection must become narrower and clearer by the exclusion of a number of border-cases as a result of the determination of its etiology.

In the case of chronic rheumatic polyarthritis — the second main clinical form of rheumatic infection — the question is however more complicated. Corresponding investigations have not given the same unanimous result here. Certainly a number of research-workers have also found here nearly the same frequency of antibodies to hemolytic streptococci as in rheumatic fever of a later stage (Blair and Hallman, Packalén), but others have recorded a considerably lower frequency (Myers and Keefer, Winblad). A conceivable explanation of this might be that the antibody production in these morbid conditions is less pronounced, but probably the chief explanation lies in the fact that what we call chronic rheumatic polyarthritis is a less homogeneous entity than rheumatic fever. The former concept connotes morbid states of different etiology but with such similar clinical features that they cannot be classified by our present clinical and bacteriological aids. Here, too, the hemolytic streptococcus seems to play an important part, possibly of various size in different countries and climates, but the future delimitation of the concept rheumatic infection, with the hemolytic streptococcus as causative agent, will probably separate a considerably larger border region here than will be the case in rheumatic fever.

The influence of tropical climate on rheumatic infection is therefore entirely consonant with its influence on the other hemolytic-streptococcal infections, but it is to be assumed that on account of what has been stated above this influence is more uncertain in the case of the present concept of chronic rheumatic polyarthritis than in the corresponding concept of rheumatic fever. The experiment made here with artificial tropical climate also argues to this effect.

To A. B. Svenska Fläktfabriken, its chief, Mr. Gustav Olsson, and other engineers, as a result of whose technical skill and financial assistance the climatic laboratory has been designed and built and the investigations rendered possible, I hereby tender my very deepest gratitude.

Summary.

In 1941 one of the wards in the Rheumatic Department of the Lund Hospital was re-constructed into a climatic laboratory so that the temperature and humidity of the air could be adjusted at pleasure between certain limits. This climatic laboratory has since been used as «a tropical chamber», that is to say, the air of the room has been kept at a constant temperature of 32° C and its humidity at 35—40 %, a relatively warm but dry tropical type. The ward contains two beds, which have been occupied by rheumatic patients during the past two years, seven with rheumatic fever and eight with chronic rheumatic polyarthritis.

The first preliminary results of these experiments are as follows:

The peripheral circulation has improved and increased in all the patients. The peripheral vasospasm has been converted into a peripheral vasodilatation. The temperature of the skin has been raised, especially of hands and feet, where at times it had been higher than on the trunk. The relative oxygen saturation of the venous blood, measured at the V. cub. med., has increased. This may also be expressed thus, that this arterio-venous difference has diminished. The diastolic blood pressure has fallen.

Broadly speaking, no changes in calorie consumption or in basal metabolism have been observed. However, there have been individual variations. Thus, the so-called second chemical regulation of temperature has not entered into function at this temperature of 32° C. in the majority of the patients.

Of the seven rheumatic-fever cases, six became entirely free from symptoms and capable of working. One died under a picture of sepsis after an acute tonsillitis (*Str. viridans*) followed by a flaming up of nephritis and uraemia.

Of the eight chronic polyarthritis cases, four have become free from symptoms and capable of working. Two cases improved

temporarily but deteriorated again after the treatment so that the final condition was not appreciable better than the initial condition. One case — a juvenile arthritis of the Still's disease type — improved very much during the first treatment but after return home had a relapse, which could not be influenced by renewed treatment in the tropical ward. In one case the treatment was broken off on account of a depressio mentis present at the same time.

In 11 of the cases — five of the rheumatic-fever cases and six of the chronic-polyarthritis cases — cultures from the throat at the commencement of tropical-ward treatment showed hemolytic streptococci. In nine of these cases, these cocci disappeared during the stay in the tropical ward. The results in this respect, therefore, agree with the corresponding results obtained by removing patients to a natural tropical climate.

The tendency to recurrence after removal back to common-room temperature has not been especially striking, only one typical recurrence having occurred.

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REVUE DES LIVRES.

von Wattenwyl: *Tierexperimentelle Untersuchungen über die Wirkung langdauernder Follikelhormonapplikation und die hormonale Tumorentstehung.* (Verlag Schwabe, Basel). (211 pages with 50 reproductions and 36 tables) 1944. Price, bound, 24 Fcs.

The author has made a great many experiments on rats, mice guinea-pigs and rabbits with large doses of oestrin. He implanted tablets subcutaneously and checked the reactions and their resorption. V. Wattenwyl could not produce cancer on rats as Lecasagne, neither could he produce cancer mammae on guinea-pigs. But he could produce precancerous epithelial proliferations, which disappeared, when the treatment had ceased. V. Wattenwyl verifies by means of test and good pictures the discoveries of Lipschütz, who was able to provoke myomata, rich in vessels, with large doses of folliculin. He warns against conclusions from animals being applied to human beings. One can not compare the large experimental doses with the therapeutic, which are small and are not given over long periods. The author chanced to find that the hair fell off during the treatment. He then studied the regrowth and could prove that it was retarded by folliculin. It became quite normal, if he stopped the treatment. He also showed, that the experimental myomata disappeared, when the treatment was discontinued.

Arnold Josefson.

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From the Karlskoga Hospital, Karlskoga, Sweden.

Intrasternal Administration of Heparin.

By

STIG LINDGREN and LENNART WALLDÉN.

(Submitted for publication March 14, 1944).

The possible administration of medicaments to the body by the intra-osseous and intramedullary route has already been the subject of enquiry. One of the first in the field was Arnold Josefson, who recommended in 1932 intra-osseous administration of liver preparations for pernicious anemia, after he had obtained good results from this type of injection. It was believed at that time that the effect of the injection was due to direct stimulation of the bone marrow.

Since then, a number of observations have been made indicating that the bone marrow and the blood stream are in intimate communication. If a medium opaque to the roentgen rays is injected into the sternum it will be seen that not only is the contrast fluid accumulated in hole-like formations in the medullary cavity but that it is also being conveyed direct from this area into the emissary veins, the *venae mammae internae*. Other observations have also proved that an injected contrast medium does not remain locally concentrated but is rapidly transported into the blood channels.

The rapidity and effectiveness with which this resorption takes place is best illustrated by the experiments which have been carried out on the intrasternal administration of large quantities of fluid, blood, and plasma. Henning (1940), and Tocantins and his collaborators (1941) have made a thorough investigation on this

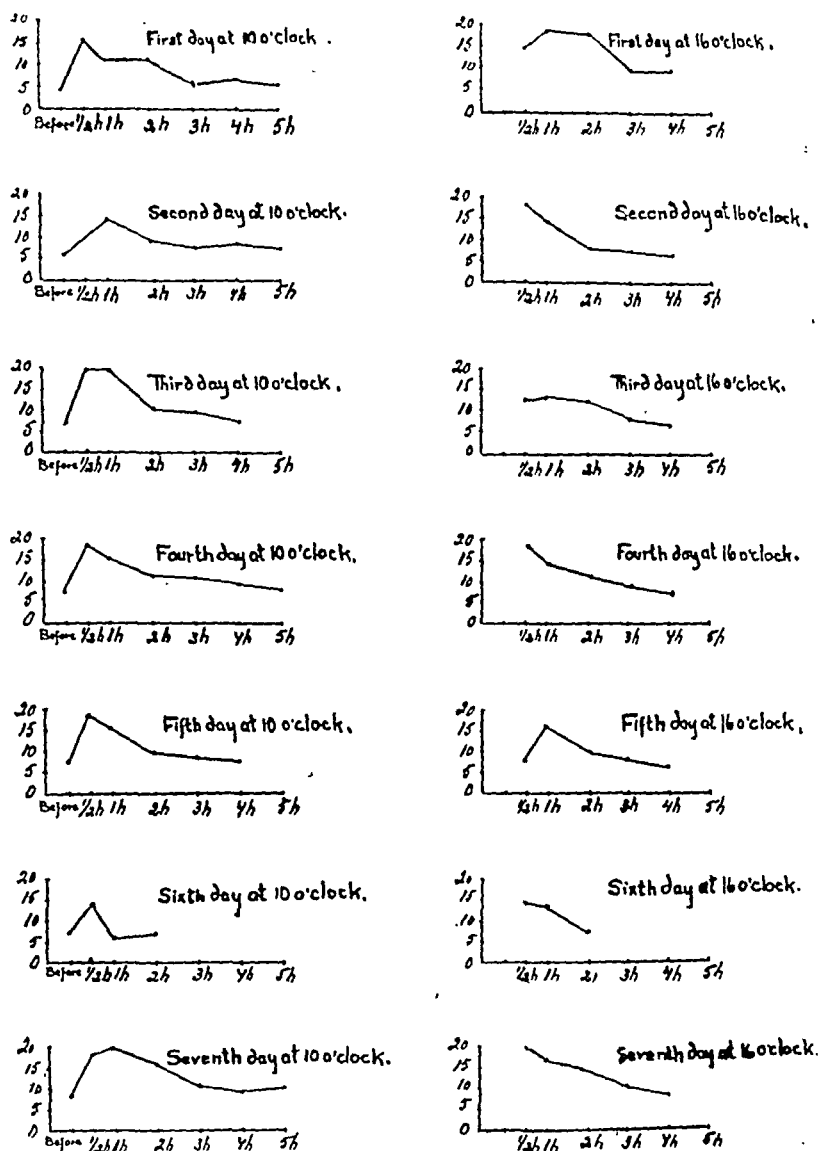


Fig. 1. Charts showing the coagulation time after the intrasternal injection of 75 mg of heparin every 6 hours for 7 days, using a needle permanently inserted in the sternum.

aspect and have reached the same conclusion, namely, that intrasternal medication can as a rule be substituted for the intravenous method.

As has been done in other hospitals, the present authors have also tried out this method of administration and have adopted it for clinical use at the Karlskoga Hospital. One liter of hemosal can be

injected within 20 to 30 minutes, a bottle of 800 cm³ of plasma in 30 minutes, and a blood transfusion in 30 minutes. The injection pressure has in these cases not been higher than that used for ordinary intravenous infusion.

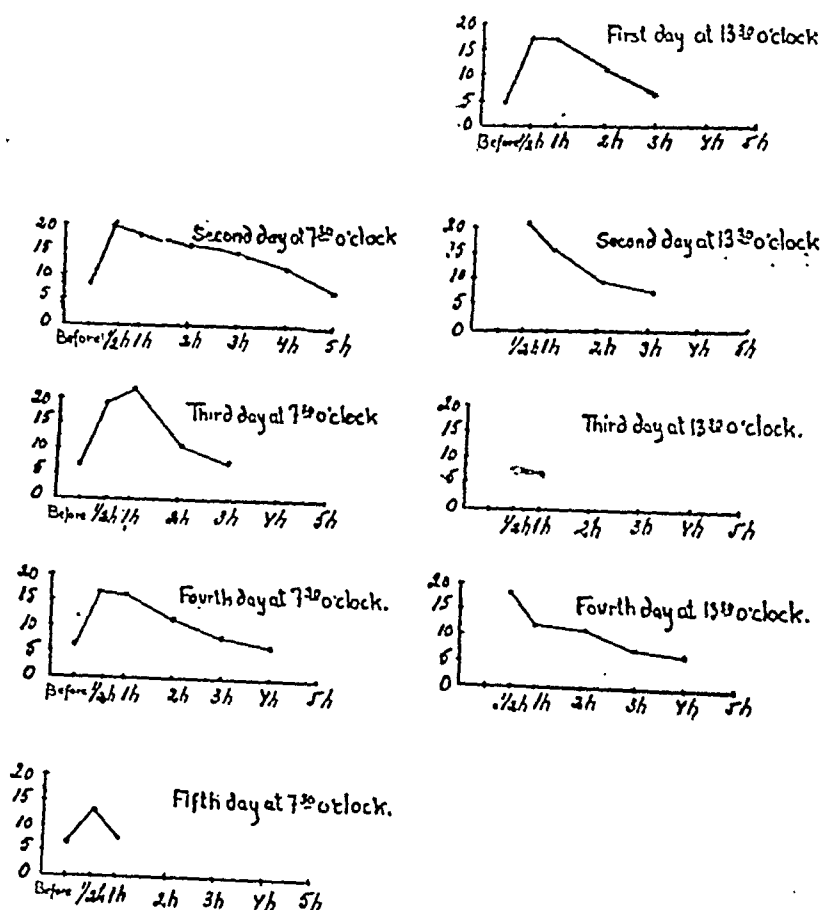


Fig. 2. Charts showing the coagulation time after the intrasternal injection of 75 mg of heparin every 6 hours for 7 days, using a needle permanently inserted in the sternum. The reason why there was no effect on one occasion was in all probability because the heparin had been injected at the side of the needle.

The advantage of this technique is that it can be used, when an intravenous infusion or injection is indicated, with patients whose veins are not easy to reach, as in the case with fat, edematous persons, and with those who have been ill and recumbent over a long period so that all their accessible veins have already been punctured; it is also useful, for instance, for patients in shock, with



Fig. 3. The sternal puncture needle in position.

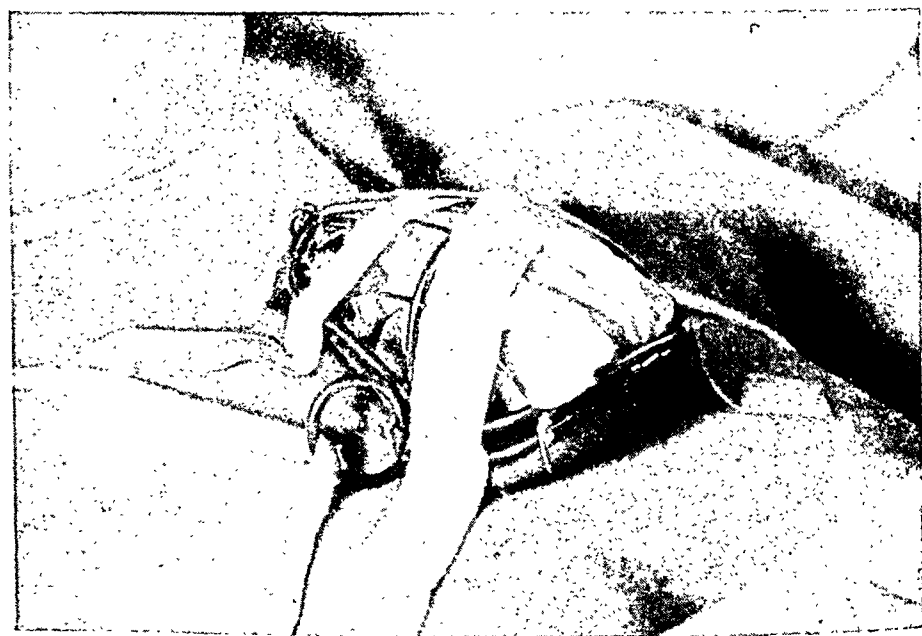


Fig. 4. This photograph shows how the needle was protected by the mask.

collapsed veins. For newborn infants, and young babies in general, the method is especially suitable, in view of the wellknown difficulty of carrying out intravenous injections in these cases. In infants, the tibia or some other fairly thick shaft bone should be

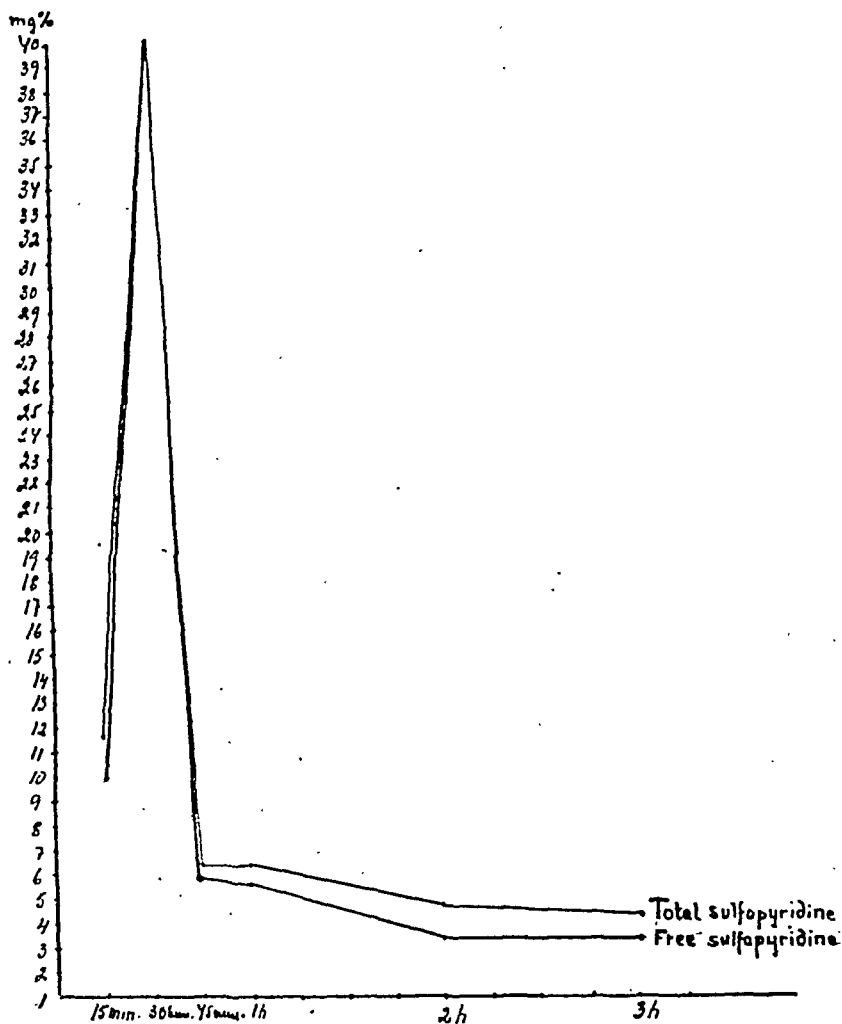


Fig. 5. This chart, which represents the concentration of sulfapyridine in the blood after injection of 10 cm³ of 20 per cent sulfapyridine, illustrates the rapid transference to the blood stream which takes place in connection with intrasternal injection.

chosen for the injection rather than the sternum. With a syringe holding 20 cm³, 40 cm³ can be injected into a newborn infant in less than ten minutes.

When thrombosis patients are treated with heparin it is not infrequently found that the veins cannot be used for the injections.

This category of patients are often obese, with veins already badly damaged by much puncturing. But heparin must be given by the intravenous route. If it is introduced by subcutaneous or intramuscular injection, it has no effect whatsoever on the coagulation time. When we tried administering heparin intrasternally we observed that *the effect on the coagulation time was the same as when it was given intravenously*. The prolongation of the coagulation time reaches its maximum within half an hour, and the effect lasts for two, three or even four hours just as it does in the case of intravenous administration. This feature is illustrated in the appended diagrams.

Technique. The instrument we have employed for the purpose is the ordinary sternal puncture needle known to all internists. It is thrust into the sternum in the same way as when samples are to be taken. It is not difficult to bring the needle into the correct position. The point ought not to be pressed too hard against the back of the bone, and care must be taken not to perforate into the mediastinum. The puncture should be made at an oblique angle, into the manubrium or the corpus sterni. Proof of the fact that the needle is in the right position can be obtained by removing the mandrin and aspirating about one-tenth of a cubic centimeter of the marrow into the needle, or by testing whether the hemosal will flow in without encountering resistance.

For heparin treatment, it is necessary that the injections should be carried out at regular intervals both day and night. It is not necessary, of course, to make a fresh puncture for each injection; the needle can remain in the sternum. This is not difficult. It lies firmly in the bone substance, and it is much easier to keep it there than it is to keep a needle fixed in a cubital vein. It should be packed with balsam of Peru and felt padding in the same way as is done with the wire at fracture extensions. The longest time we have kept a needle in the sternum is one week. But by that time the skin was red and tender around the needle. No serious infection has occurred in connection with our cases, however. One week is perhaps unnecessarily long to keep the needle inserted in the same position, as it can so easily be removed from the manubrium to the corpus sterni, and vice versa. In order to protect the needle from knocks, we generally make use of a steel-frame mask of the kind commonly used for administering anesthetic

tics. This is arranged over the needle like a hood and held in place with sticking plaster.

The diagrams show the data obtained from cases in which 75 mg of heparin had been injected every six hours during the entire 24 hours, in connection with which the prolongation of the coagulation time was determined at certain intervals. This effect appeared with the same regularity as in the case of intravenous administration.

The method is recommended as an improvement in technique.

Summary.

The intrasternal method of administering heparin produces the same effect on the coagulation time as intravenous injection.

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From the Med.-epidem. Dep. A of the Marselisborg Hospital, Aarhus (Denmark). (Chief Physician: Gregers Norby, M. D.)

Paroxysmal Paralytic Hemoglobinuria.

By

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(Submitted for publication February 10, 1944).

The disease which will be mentioned in the following is an extraordinarily rare lesion, as only 4 cases have been mentioned in the literature. None of these publications came from the Scandinavian countries. Through the differential-diagnostic considerations concerning the case to be reported here and through its possible relation to certain muscular dystrophies, however, this case appears to offer considerably greater prospects than might be expected from the infrequency of the disease, and hence a detailed report of this case will seem appropriate.

The disease belongs to the so-called *hemoglobinurias*, which in the wider sense of the term means the appearance of free hemoglobin in the urine, which in these conditions usually is from dark brown to reddish in color. This group of lesions was first differentiated from the hematurias proper by Ponfick in 1883; and, as is well known, in practice the simple criterion of differential diagnosis is observed: in hemoglobinuria the urine gives a strong reaction for blood in the chemical test, while on microscopy it shows but few red blood cells or none at all.

Hemoglobinuria may be found under various morbid conditions, the more important of which are listed in Table 1.

Table 1.

*Survey of Occurrence of Hemoglobinuria.*A. *Exogenous:*

1. *Infections:* Typhoid fever, malaria, scarlet fever, sepsis, puerperal infection with *Bac. perfringens*.
2. *Poisoning:* Chlorates, phenols, pyrogallol, quinine, sulfanilamide and its derivatives.
3. *After blood transfusion.*
4. *»Haff disease».*
5. *Favism.*

B. *Endogenous:*

1. *Cold-hemoglobinuria.*
2. *March-hemoglobinuria.*
3. *Chronic hemolytic anemia (Marchiafava type).*
4. *Paralytic hemoglobinuria (myoglobinuria).*

The last two subgroups of the exogenous hemoglobinurias are to be mentioned briefly, as they have not been dealt with previously in the Scandinavian literature.

»Haff disease» (Haffsucht, Haffkrankheit) is named after the large shore-lakes (Haffe) found in the vicinity of Königsberg, in East Prussia, and in this connection, especially Frisches Haff. Large cellulose factories are located here, the waste-water of which is led out into the Haff; and this waste-water contains large amounts of poisonous pitch compounds. Certain fishes (especially eel), living in the haffs, get sick in this milieu; and when the fish is caught and eaten by the local inhabitants, the people become ill with attacks of muscular pain, stiffness, difficulty in walking and hemoglobinuria, which on further examination is found to be due to excretion of muscle hemoglobin. If the illness terminates fatally, pronounced degenerative processes are found in the striated musculature; which is pale, reminding of the flesh of fish. As will be evident from the following, in several respects this disease reminds of paralytic hemoglobinuria.

The last of the exogenous hemoglobinurias entered in Table 1, *favism*, is named after the bean *Vicia fava*, which is particularly common in the Mediterranean countries, especially Sicily and South Italy. It has now been established that inhalation of the

pollen of this plant or intake of its beans makes people ill with lassitude, fever, rapidly developing anemia and jaundice, besides abdominal pain and hemoglobinuria. The affection is assumed to be due to an allergic reaction elicited by proteins in the plant concerned; and it appears as if the persons affected in this way have to be sensitized beforehand. So this form of hemoglobinuria may be looked upon as a transition to the other large group — the endogenous hemoglobinurias — all of which as a rule are paroxysmal in their appearance.

In this group *cold-hemoglobinuria* is the most well-known, etiologically as well as pathogenetically. The characteristic feature of this form is that the patient has attacks of pain in the back and over the loins, rise in temperature, sometimes with chills, and hemoglobinuria. On more thorough examination of the patient he is nearly always found to be syphilitic (over 95 % of the patients give a positive Wassermann reaction), that the attacks are preceded by chilling of the body, and that the attacks may be provoked by the chilling of an extremity (hand, foot) placed in ice-water for a few minutes. In the serum of these patients Donath & Landsteiner have demonstrated the presence of an agglutinin which in cold — *i. e.*, in the peripheral parts of the body on exposure to cold — unites with the red blood cells of the patient, and at the higher temperature in the inner parts of the body produces hemolysis (in the presence of complement). The affection is cured by antisyphilitic treatment.

The next form, *march-hemoglobinuria* (hemoglobinuria from exertion), is far more infrequent, as only twenty odd cases have been reported in the literature. It appears especially in soldiers after long marches. It differs from the preceding form in the absence of syphilis in the history of the patient, in not being elicited by exposure to cold (whereas it is provoked by physical exertion, especially in upright posture), negative outcome of the chilling test and negative Donath-Landsteiner reaction. Possibly the degree of the lumbar lordosis plays an etiological role, making this affection somewhat related to orthostatic albuminuria. The pigment in the urine is blood hemoglobin. The prevailing view is that this form of hemoglobinuria is not a result of pronounced intravascular hemolysis but more likely limited to the renal vessels, and perhaps brought about by the lumbar lordosis. The disease is

quite harmless; the patients recover spontaneously — without any active treatment.

The next subgroup is a form of hemoglobinuria due to a special hemolytic anemia, which was described first by Marchiafava in 1911 and is characterized by the following features: 1) hemolytic non-hereditary anemia, often accompanied by jaundice; 2) paroxysmal pains in the back and abdomen, often accompanied by fever; 3) recurrent nocturnal appearance of hemoglobinuria. Cases have been reported from Sweden by Bergmark and Salen, from Denmark by Arndal and Brendstrup.

The last form entered in Table 1 is the most infrequent of all the hemoglobinurias: the *paroxysmal paralytic hemoglobinuria* or, more correctly, *myoglobinuria*, as will be evident from the following. This form of hemoglobinuria appears as paroxysmal pains in the legs, loss of muscular power increasing to paresis which may subside or lead on to death. During the attacks the urine is dark reddish-brown owing to hemoglobinuria. Extensive degenerative processes are found in the muscles. The disease has a tendency to remissions.

As mentioned in the introduction, only 4 cases of this disease have been reported so far, on which account it will be reasonable here to review these cases. The first description of the disease was given in 1911 by Meyer-Betz, of Munich, in an excellent paper with a thorough and detailed account of the new disease, so that the following reports added no new essential feature to the nosographic picture of the lesion.

Pt. No. 1 (case reported by Meyer-Betz in 1911):

The patient was a boy, 13 years old. At the age of 9 years, a brief period with muscular weakness and black urine. In the following year (1907) a similar attack. As examination revealed atrophy of the musculature of the shoulder girdle, while the gastrocnemii were large, tense and hard, progressive muscular dystrophy was diagnosed. Two years later, again a severe attack accompanied by abdominal pain and pronounced pareses. The urine was bloody for 2 days. The diagnosis was muscular dystrophy and hemorrhagic nephritis, till the author demonstrated that hemoglobinuria was involved, assuming it to be an instance of methemoglobinuria. The boy, who at one time was moribund, recovered. The pareses subsided completely, and at the discharge of the patient there remained only a slight contraction of the Achilles tendon on one side.

The next case was reported in 1924 by Paul, of Vienna.

Pt. No. 2 (case reported by Paul in 1924).

The patient was a woman, aged 42, who suddenly became ill with chills, fever, pain in the extremities and abdomen and reddish-brown urine, due to hemoglobinuria. Later the clinical picture was dominated by diarrhea and increasing pareses of the extremities. The patient died on the 15th day of illness. Autopsy revealed extensive wax-line degenerative changes in the musculature (extremities, diaphragm, abdomen). The muscles involved reminded of the flesh of fish. Histological examination showed fragmentation of the muscle fibres, considerable growth of the interstitial tissue and involvement of the perimysium. In several areas heaps of delicate and faintly staining muscle fibres were seen, characterized by the presence of numerous swollen nuclei poor in chromatin — which changes were interpreted as products of a faint tendency to regeneration.

Also Case 3, reported by Günther of Leipzig in 1924, terminated fatally.

Pt. No. 3 (case reported by Günther in 1924):

The patient was a man, 54 years old, who suddenly became ill with an influenza-like lesion associated with fever, chills, dedolations and weakness. 3 weeks later the urine was dark-colored, and now extensive pareses appeared, so that the patient was unable to move about. He died with signs of cardiac insufficiency. Examination of the urine showed hemoglobinuria; but the author stated that the color of the urine was hardly due to blood pigment but presumably to muscle pigment. On autopsy the muscles were found to be very pale; and microscopic examination showed extensive degenerative processes in the muscles.

The fourth case was reported in 1925 by Hittmair, of Vienna.

Pt. No. 4 (case reported by Hittmair in 1925):

This was the case of an adult woman which was of particular interest because the patient gave a family history of disposition to this affection. Her brother had had similar attacks — once in childhood and 5—6 times in youth — with lassitude, indisposition, pains in the extremities and dark urine. During the attacks he was unable to move about.

Apart from attacks of migraine, the patient gave a past history of good health. In 1923—24 she had 4 attacks of her present illness with dark urine and pareses. Each attack was acute in onset and took a serious course. It appeared as if muscular exertion had a provocative effect. Abdominal discomfort and muscle pains were very pronounced symptoms. The muscle pain and the pareses commenced in the lower extremities but extended up to the occipital musculature, whereafter they regressed. Spectroscopic examination of the urine showed the presence of oxy- and methemoglobin, but the author assumed it was a question of excretion of muscle hemoglobin.

Besides describing their cases, the four above-mentioned authors also discussed the character and nature of the disease. Meyer-Betz considered thoroughly its relation to progressive muscular dystrophy, to which the lesion in his case presented a striking resemblance. Still, he did not think the two diseases were identical, and he considered it most likely that in paralytic hemoglobinuria it is a matter of a noxious substance, toxic or infectious, which attacks both the blood and the musculature. Paul subscribes in part to the view advanced by Meyer-Betz, and he likewise points out certain analogies to the paralytic hemoglobinuria in horse (see below), with which he classifies the disease, calling it myopathic hemoglobinuria. Hittmair and Günther added nothing fundamentally new in their considerations concerning this lesion but interpreted it as a sudden intoxication of obscure origin with damage to the musculature.

In the veterinary pathology, as mentioned, there is a not altogether infrequent disease — paralytic hemoglobinuria in horse — which greatly resembles the paralytic hemoglobinuria in man, on which account it will be mentioned briefly here (for literature, see the works published by Carlström, Sweden). Equine hemoglobinuria occurs especially in horses which have been standing in the stable for 1—2 days (as often is the case during week-ends) and in which time they have been fed very well. When such animals commence working again they may have stiffness and soreness corresponding to the musculature of the hind legs and loss of muscular power increasing to complete paresis, so that the animal breaks down. The urine is dark red, and recently this color has been demonstrated to be due to the appearance of muscle pigment in the urine. The disease may be quite transitory, lasting only a few days, but it has a tendency to relapse. In other cases the affection extends to involve other muscle groups too, terminating fatally; and autopsy reveals pronounced degenerative changes in the muscles.

In the following an account will be given of our own case.

Pt. No. 5 (writers' case):

This patient is a boy, 10 years old, who was admitted on 12/7/43 for observation for progressive muscular dystrophy.

In the mother's family there are said to have been several cases of a

»muscular affection» of disabling character (disposition will be taken up later).

The boy was perfectly well till the age of 4 years, whereafter he has had repeated attacks of muscle pain and difficulty in walking. When running about playing he sometimes suddenly would stop and cry, complaining of pain in the legs. During such attacks it was impossible for him to walk; and the parents noticed that during the attacks, which as a rule were very brief and transitory, his calves were thick, firm and tender to touch. These attacks of muscle pain and loss of muscular power have since recurred not infrequently. The mother further stated that during the attacks the urine had occasionally been bloody — which information we did not pay particular attention to at first.

Physical examination revealed no abnormality except that the musculature of the calves perhaps was rather robust. The boy looked healthy and normal; mentally he presented no particular abnormality. Neurological examination showed normal conditions, no atrophy was found and the muscular power was good.

Of the laboratory tests it will suffice here to mention that Wassermann was negative, sedimentation rate 4 mm, hemoglobin 99 %, serum calcium 10.6 mg %, and the urine contained no sugar or albumin.

In order to obtain information about the boy's capacity for work and power of endurance, we let him work on a bicycle ergometer: 360 kg per min. — a not inconsiderable amount of work for a boy of his age. He did this work in 20 minutes without any particular inconvenience.

Thus our examination of the boy turned out rather negative but with a view to the family disposition to muscular affection, together with the pain in his legs and slight hypertrophy of the musculature of the calves, the diagnosis recorded at his discharge on 21/7/43 was: Incipient progressive muscular dystrophy? As mentioned above, this was also the diagnosis first made in the case reported by Meyer-Betz.

3 weeks after the discharge, the father returned to the hospital with the boy. The boy had just commenced school, when on his way to the school baths he suddenly became ill with intense pain in the legs; and, like before, he had difficulty in walking. These phenomena became worse, when the boy reached the swimming pool where he hid himself because he was unable to walk home. The father found him a couple of hours later rather exhausted, complaining of pain in the legs, and then the father brought him directly out to the hospital. Now the boy gave an impression of marked exhaustion; he was pale and chilly; and he complained of pain in the abdomen and in the legs. His gait was very laborious, waddling, and it was plainly noticeable that walking was associated with pain. The gastrocnemii were tense, tender and considerably more voluminous than on his first admission; the patellar reflexes were a little weak. The boy returned to his home and was put to bed. On the following day the family physician sent us a specimen of dark reddish-brown urine, which the boy had voided. The urine gave strong reactions for albumin and blood; but no red blood cells were found on microscopic examination of the urine,

i. e., this was an instance of hemoglobinuria, on which account we advised hospitalization of the patient; and he was readmitted from 14/8—23/8/43.

The boy had now got over his attack, the urine was again light in color, and we found nothing new on physical examination. We were now inclined to combine the hemoglobinuria with the previous diagnosis of progressive muscular dystrophy, assuming that there might be an instance of myoglobinuria, as we found this disease described in the literature. Before mentioning why we decided on myoglobinuria, however, it will be appropriate briefly to mention the outcome of the ordinary laboratory tests.

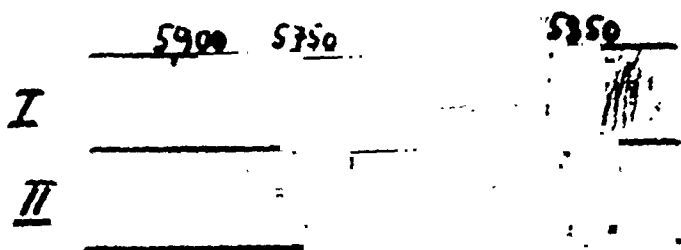


Fig. 1. Absorption bands in myoglobinuria.

Spectra from:

- I. Urine of the patient with myoglobinuria.
- II. Blood of a normal subject.

Hemoglobin 100 %. Red blood cells 4.32 mill. White blood count 6200. Platelet count 150,000. Reticulocytes $\frac{1}{2}$ %. Plasma color 5 (Meulengracht). Osmotic resistance: Beginning hemolysis at 0.44 % NaCl; total hemolysis at 0.35 % NaCl. Differential count: Neutrophils 43 %, lymphocytes 51 %, monocytes 6 %. Cold test, negative. Donath-Landsteiner agglutinin: 0 (the test was kindly performed by Dr. P. V. Marcussen, the State Serum Institute). Blood group: B—M.

With a view to the spectroscopic examination we had collected the urine from the first days of illness and stored the portions in a refrigerator. The urine voided during the attack, as mentioned, was dark reddish-brown, reminding of port wine. On the following day the urine was already noticeably lighter in color though still dark brownish. Three days after the attack the urine was normal in color (light yellowish).

The outcome of the spectroscopic examination is presented in Fig. 1¹. Suitable dilutions were made of hemolysed blood from a normal person (in Fig. 1 designated as II) and of the patient's urine (in Fig. 1 designated as I), and at the examination it was

¹ This examination was performed in the Chemical Institute of the University with the kind assistance of Professor Haakon Lund, Ph. D. and Professor F. Schonheyder, M. D. We wish to acknowledge our indebtedness for this valuable assistance, without which we should not have been able to carry through this examination.

practicable to observe the two spectra at the same time and thus compare them easily. In Fig. 1 the two characteristic bands for oxyhemoglobin will be noticed; the bands for the diluted urine remind greatly of this picture but are shifted somewhat to the left, i. e., towards the red field in the spectrum. On measuring,

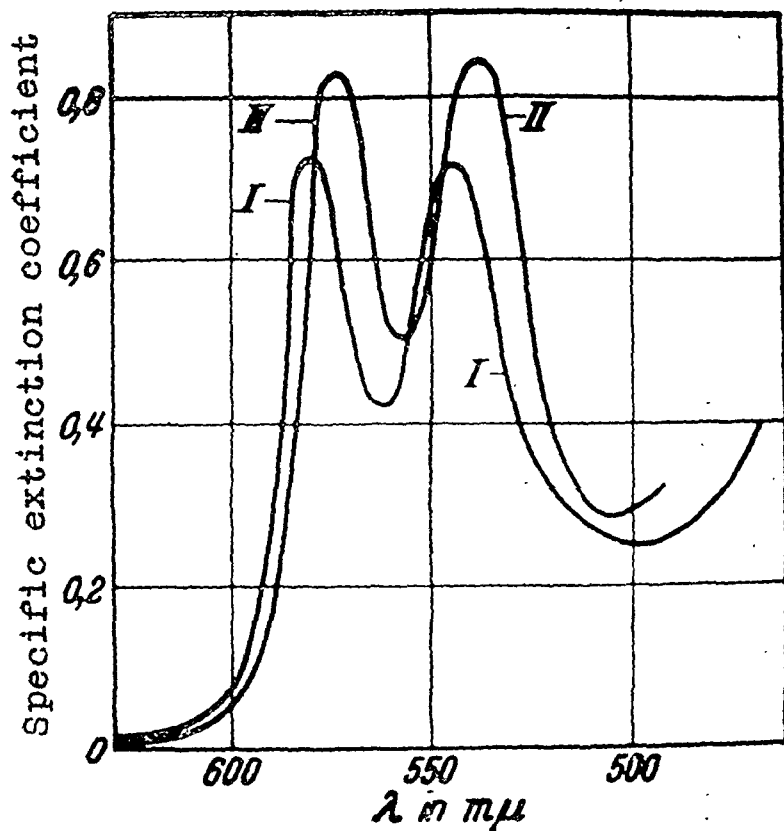


Fig. 2. I. Oxymyoglobin.

II. Oxyhemoglobin.

Ordinate: Extinction coefficient.

Abscissa: Wave length in Å. U. (after Theorell).

this shift was found to be about 50 Ångström units, their location being about 5750 and 5350 Å. U. As is evident from Fig. 2, these features are just characteristic of myoglobin.

Fig. 2 is reproduced after a work by Theorell, Sweden, whose particular accomplishment has been to define the molecular weight spectrum, oxygen combining curve and other aspects of myoglobin. The two curves for oxymyoglobin and oxyhemoglobin resemble each other a good deal, but in comparison with oxyhemo-

globin the apices for oxymyoglobin are shifted somewhat to the left, and for myoglobin the apices (corresponding to the location of the absorption bands) are seen at 5750 and 5350 Å. U. — just as observed on spectroscopic examination of the patient's urine. So there could be no doubt that here we were dealing with an instance of *myoglobinuria*.

Of other examinations, mention is to be made of the behavior of *creatinin* in the urine. The *creatinin* determinations on the urine are recorded in Table 2.

Table 2.

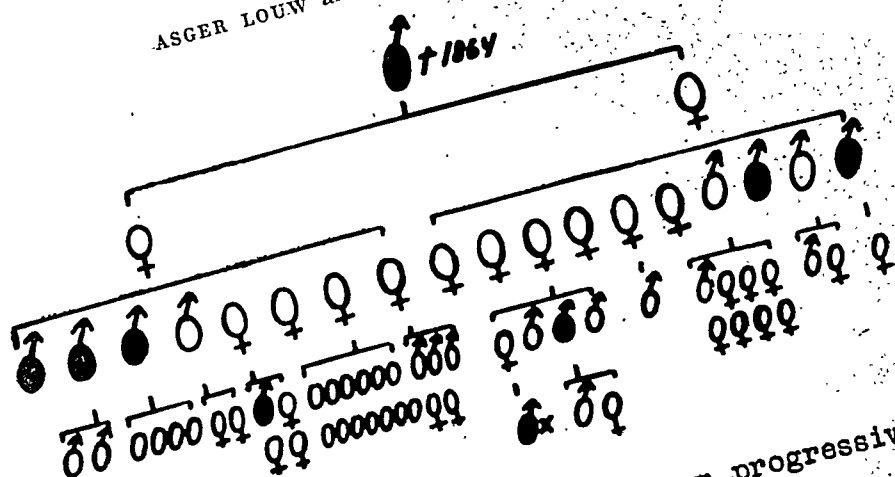
Creatinin Determinations on the Urine of a Patient with Myoglobinuria.

12/8:	Preformed creatinin	0.947 g	per 1000 cm ³			
	Total	»	2.335 g	»	»	»
15/8:	Preformed	»	0.942 g	»	»	»
	Total	»	0.925 g	»	»	»
18/9:	Preformed	»	1.20 g	»	»	»
	Total	»	1.19 g	»	»	»

As is well known, the difference between the total and the preformed amounts of *creatinin* gives the *creatin* excretion, and normally the total and the preformed amounts are identical, *i. e.*, there is no *creatinuria*. From Table 2 it is evident, however, that during the attack there is a rather considerable *creatinuria*, which soon disappears and is absent outside the attack. *Creatinuria* is known to occur in a number of different morbid conditions but is encountered above all in various muscular and neuromuscular lesions — in particular, in patients suffering from progressive muscular dystrophy. It was only natural, then, that this temporary *creatinuria* in our patient made us even more interested in the muscular affection which was said to occur in the mother's family.

With the assistance of general practitioners, hospitals and members of the family we succeeded in obtaining a pedigree of the family, which was scattered all over Jutland, though mostly Western Jutland. The pedigree which is presented in Fig. 3, comprised five generations; and from this chart it will be noticed that the family included 8 cases of progressive muscular dystrophy — besides our case of paroxysmal paralytic myoglobinuria. Only males had this affection, but women did appear as conductors, *i. e.*, the inheritance was recessive and sex-linked. The progenitor

ASGER LOUW and HOLGER E. NIELSEN



Dystrofia musculorum progressiva
Paroxysmal Paralytic Myoglobinuria

Fig. 3. Pedigree of family with progressive muscular dystrophy (black spots) and paralytic myoglobinuria (x).

had immigrated from Sweden, and the only information obtainable about him was that he was suffering from a muscular affection localized to the lower extremities, and that he died of this lesion at a relatively young age. His two daughters presented no abnormality but — as is evident from Fig. 3 — were conductors. Among their 18 children 5 were suffering from a muscular affection which, in all of them, commenced in childhood or at the time of puberty. The lesion manifested itself in pain in the legs, loss of muscular power and atrophy of the proximal muscle groups. Two of the men in this generation died at a relatively young age, respectively 43 and 41 years. In the fourth generation 2 men were suffering from the muscular affection, which in both cases commenced at the age of 14—15 years with pain in the legs, loss of muscular power, difficulty in walking and atrophy of the proximal muscle groups. Besides, in both of these patients the calves of the legs were very well-developed, forming thus a striking contrast to the rest of the musculature of the legs which had undergone atrophy. All these features were quite in keeping with progressive muscular dystrophy, especially the hypertrophic type, in which the inheritance has been described exactly as the one observed in this family: sex-linked recessive. All these patients gave the same history of ab-

sence of previous periods with dark or black urine — such as observed in our patient.

Naturally the occurrence of our case of paroxysmal paralytic myoglobinuria in this family with progressive muscular atrophy raises the question: Are these cases different forms of the same disease? For elucidation of this question, in Table 3 we have entered the more important features of the two lesions for comparison.

Table 3.

	<i>Paralytic myoglobinuria</i>	<i>Progressive musc. dystrophy</i> (hypertrophic type)
Onset	sometimes in childhood	in childhood
Course	acute, with remissions	chronic
Muscles attacked	proximal groups (hypertrophic calves)	proximal groups (hypertrophic calves)
Creatinin output	periodical creatinuria	permanent creatinuria
Hemoglobinuria	+ myoglobinuria	— (?) myoglobinuria
Pathological anatomy	Pale muscle fibres. Deterioration of the contractile substance. Indistinct striation. Degeneration, fragmentation of muscle fibres with increase in number of nuclei.	Pale atrophic muscles. Increase in the interstitial con. tissue and fat. Indistinct striation. Degeneration, fragmentation of muscle fibres with increase in number of nuclei.

From Table 3 it will be noticed that there is a great deal of resemblance between the nosological findings in the two conditions; and the idea suggests itself that the etiology of the two lesions may be the same: some abnormality in the muscular metabolism of creatinin, which in patients with paralytic myoglobinuria is active only for a *short time*, giving an *acute* lesion associated with injury to the musculature which may recover partially between the attacks, whereas the abnormal process in patients with progressive muscular dystrophy is *permanently active*, giving rise to a chronic lesion with pronounced and permanent degeneration of the muscles.

It will be of interest in future cases of progressive muscular dystrophy and paralytic myoglobinuria to examine the families with reference to these features.

Summary.

After a survey of the more frequent causes of hemoglobinuria, brief mention is made of the 4 cases of paroxysmal paralytic hemoglobinuria (or, more correctly, myoglobinuria) reported hitherto.

An additional case of myoglobinuria is reported in a boy 10 years old, with a history of repeated attacks of muscular pain and difficulty in walking since the age of 4 years.

During an acute attack, the boy was admitted to the hospital with intense pain in the lower extremities and unable to walk. During this attack the urine was dark, reddish-brown and gave a strong reaction for blood, but showed no red blood cells on microscopic examination.

Spectroscopic analysis of the urine showed that in this case myoglobin was excreted with the urine during the attack, at which time there was also a marked creatinuria.

Myoglobinuria and creatinuria were demonstrated only in connection with the attack, which soon subsided without leaving any paresis.

The boy was feeling perfectly well at his discharge from the hospital.

The appearance of 8 cases of progressive muscular dystrophy in the family of this boy suggested the hypothesis: that progressive muscular dystrophy and paralytic myoglobinuria may be different forms of the same disease, the latter representing the acute manifestation of the lesion, while progressive muscular dystrophy is the more chronic form of the disease.

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(From the Biological Laboratories of Medicinalco, Ltd. Copenhagen, S).

Some Investigations on the Content of Reticulocyte-Ripening Substances in Various Organs.

By

CLAUS MUNK PLUM.

(Submitted for publication February 14, 1944).

When stained supravitaly with basic dyes some red blood corpuscles show a »reticulated substance» consisting of fine or coarse granules connected with each other by a fine filament. Such erythrocytes are called *reticulocytes*. In the blood of normal animals between 0 and 30 per mille of the total number of erythrocytes are found to be reticulocytes varying with the species and the age of the animal examined. Now the reticulocytes generally are accepted as young red blood cells. Whenever the blood formation in the bone marrow is more active the number of reticulocytes in the blood increases. This phenomenon has caused the importance of reticulocytes in the clinic where extensive studies have been carried out on this subject. Pure physiological investigations on reticulocytes are rather scanty and our knowledge here limited.

When blood is stored the reticulated erythrocytes gradually disappear. This was shown for the first time by Pepper (1922) who incubated human or rabbits blood stabilised with citrate at body temperature while blood stored at 4° showed no alteration in this respect. The same was seen by Seyfahrt (1927). Heath & Daland (1930) found that reticulocytes in vitro at 37° or in the pleural cavity of the rabbit could after a few days no longer be identified by supravital staining, decreasing at a regular rate from the first day. The rate was similar for reticulocytes from rabbits which had been

bleed or made anemic with phenylhydrazin, and from patients with hemolytic jaundice or pernicious anemia. The rate was slower at temperatures and reticulocytes persisted in blood kept in the ice box for six months. Krafta (1931) concluded that a temperature coefficient for the loss of specific staining qualities in animals after death is indicated and is of the order of that for chemical reactions. The mechanism of this disappearance of the reticulated cells has been the subject of several investigations though merely from a morphological point of view.

The amount and the appearance of the reticulum in the cells varies. Cesaris-Demel (1907) found different types of reticulocytes according to the amount of stainable substances in the cells. Investigations of Rosin & Bibergeil (1904) Seyfahrt (1927), Seyfahrt & Jürgens (1928) Moldawsky (1928), Gawrilow (1929), Riddle (1930), Heath & Daland (1930), showed that the types containing much of the substance which aggregates in the form of a reticulum are to be regarded as younger cells while the cells containing lesser amounts are to be regarded as older stages of the reticulocytes. The comparative age of the reticulocytes can be estimated from the stained film, the heavily clumped and wreathed forms being the most immature and the sparsely reticular and punctate forms, the older cells. Riddle (1930) estimated from investigations on patients with pernicious anemia that reticulocytes with very little reticulum material disappear within 24 hours, but that younger reticulocytes differentiate into mature erythrocytes in from two to four days, depending on the intensity of the stimulus to regenerating.

Experiments with blood incubated in vitro show that first the older forms disappear and later the younger forms, Heilmeyer & Westhäuser (1932). Both Pepper (1922) and later on Heath and Daland (1930) found that the reticulocytes were not replaced by structureless adult erythrocytes but by cells containing stainable granules. The cells mentioned by Pepper (1922) and Heath & Daland (1930) must, however, be regarded as the oldest stage of the reticulocytes; and the results of the countings by Heilmeyer & Westhäuser (1932) show that even this type of reticulocytes disappears when the blood is incubated long enough.

Furthermore it is shown that all erythroblasts containing hemoglobin are heavily loaded with reticulum. From this fact and

from the investigations mentioned it must be concluded that the reticulocytes are unripe red blood cells which, from types with clumped reticulum through types with decreased amounts ripen into mature erythrocytes.

Recapitulating our knowledge of the ripening process of the reticulocytes it is stated: 1) that the reticulocytes ripen through more mature types into normal «adult» erythrocytes, 2) that the ripening process even goes on *in vitro*, and 3) that it is accelerated with increasing temperature.

In a series of investigations previously reported (C. M. Plum, 1942) it was proved that certain substances were present in the plasma as well as in liver extracts which were able to influence (accelerate) the ripening of reticulocytes *in vitro*.

Jacobsen & Plum (1942) have shown that the ripening substances occurring in liver extract may be divided into two fractions, 1) a thermostable factor, little active by itself, which has been identified as tyrosine, and 2) a thermolabile factor, of little effect in itself, the chemical nature of which is still under investigation. Further it was demonstrated that tyrosine in slight quantities was able to accelerate the effect of the thermolabile factor, just as it was found that the addition of tyrosine to a liver preparation could increase the reticulocyte-ripening power of that substance.

Further Jacobsen & Plum (1943) found that blocking of the reticulo-endothelial system in rabbits will cause an immediate reduction in the amount of reticulocyte-ripening substances in the plasma of the animals. The addition of tyrosine to this plasma has the effect of raising to normal values the power of ripening the reticulocytes.

Thus the plasma of treated rabbits can be activated by tyrosine in contrast with the plasma of untreated rabbits, while at the same time the ripening activity has decreased. From this Jacobsen & Plum conclude that the reticulocyte-ripening principle consists of tyrosine or a tyrosine-like substance and an unknown thermolabile factor which are linked together by the co-operation of the reticulo-endothelial system.

So far the reticulocyte-ripening substance has been found to be present in plasma and in liver extract (e. g. Hepsol fortior «MCO»). The investigations reported in the present paper comprise

the demonstration of the presence in other tissues and organs of reticulocyte-ripening substances.

The investigation falls into two groups, viz. 1) the demonstration of substances activating the ripening of reticulocytes in-vitro without the addition of tyrosine, and 2) experiments with extracts of organs in which the amount of fully ripened substances was determined, while it was also ascertained whether substances were found in the extracts which after the addition of tyrosine were able further to activate the ripening of the reticulocytes.

Substances acting without the addition of tyrosine are called *mature ripening substances*, and substances that become active only after the addition of tyrosine are termed *preformed ripening substances*.

The organs examined were chosen according to whether they were active or inactive with regard to erythropoiesis; in the first group were included the liver, bone-marrow, spleen and stomach, and in the second the brain, heart, ordinary musculature, kidney and for some animals also the thymus.

I. Investigations on the expressed Juice.

For these investigations were used organs from ox, pig, and in the case of some organs from rabbits.

Production of the expressed juice. Immediately after the organs had come from the slaughterhouse, or after the animal had been killed in the laboratory, the organs were cut up and all macroscopical blood coagulates removed. After this they were finely minced by passed through a mincing machine. The minced organs were then stirred up with dry clean sand and annealed kieselguhr to a »dry» porridge, which was placed in a Buchner press. The juice was expressed under a pressure of 400—500 atmospheres. For most of the organs there was little difficulty in squeezing out the juice, to which immediately after the expression a few drops of »Solbro» solution were added, whereupon it was put in an ice-box till the next day (about 16 hours). Pressing out the bone-marrow juice was rather difficult, and the expressed fluid was a viscous oil-like liquid not at all well suited for analysis. After it had been stored in the ice-box the fat had set and could easily be removed, and the remaining fluid could be used for the experiments.

The analysis of the content of ripening substances in the expressed fluids was then carried out according to the method devised by C. M. Plum (1942). For the technique the reader is referred to previous reports.

As the expressed juices were often turbid they were centrifuged immediately after the experiments had been started, but in spite of this particles were found which would take a cresyl blue stain and which, when they settled on the erythrocytes in the preparations, often made these resemble reticulocytes, just as large precipitates were often found which would likewise stain. The results of these investigations are therefore beset with a not inconsiderable incertainty.

For technical reasons the samples had to be left for almost 24 hours before they were examined, but as a check on the possible wasting of the ripening substances while stored a single experiment was made immediately after the expression, and in this it was found that the wasting of the ripening substances was very slight.

The result of these investigations will appear from Table 1.

II. Investigations on Extracts of dried Organs.

For these investigations the organs of ox, cat, pig, dog, rabbit, guineapig, rat and mouse were used.

Making of the extracts. Immediately after the organs had been received or the animals had been killed in the laboratory the organs were cleaned and the macroscopic blood coagulates removed, after which they were minced and placed in an incubator (Max. temp. 42°), until they were dry, i. e. for 24 hours. The dried organs were pounded in a mortar. 1 g of the powder was added to 10 cm³ 0.9 % NaCl-solution, carefully mixed and the mixture placed in an ice-box till the next day (about 16 hours). This storage time was strictly adhered to, as it turned out that lower values were found (about 25 % wastage) if the solution was left for 2 days. Immediately before the experiment the solution was stirred up after which it was centrifuged.

The method of determination was the same as for the determination of the content of ripening substance in the expressed juice, with the difference only that here partly the pure extract and partly the pure extract with the addition of tyrosine were tested.

The results will appear from Table 2.

Table 1.

Ripening index of expressed juice for various organs derived from different animals.

	Ox				Pig			Rabbit (untreated)		Rabbit (treated)	
	Ripening index				Ripening index			Ripening index		Ripening index	
Plasma	1.00 ¹				0.80 ²			0.71 ³		0.93	
Brain	0.17		0.19	(0.20)	0.08						
Heart	0.28										
Striated muscle	0.21	0.22	0.24	(0.23)	0.18	0.16	0.15	0.12	0.10	0.17	0.19
Thymus		0.15	0.16	(0.17)	0.09		0.10				
Lung	0.13		0.11	(0.13)	0.10						
Liver	0.34	0.40	0.38	(0.41)	0.30	0.28	0.32	0.21	0.18	0.29	0.32
Spleen	0.47	0.45	0.42	(0.45)	0.29	0.26	0.32				
Bone marrow		0.37	0.39		0.26		0.29				
Stomach					0.11		0.12				
Stomach with- out pylorus ..					0.09		0.08				

Discussion.

If the results are compared it will be seen that in the animals examined there is a close correlation between the amount of ripening substances in the plasma and in the organs, animals with a high content in the plasma having relatively more than animals with a low content in the plasma.

The examination of the expressed juice furnished information as to the distribution of the ripening substances in the organism. We here refer to the ripening substances that do not require the further addition of tyrosine to be demonstrated; they are here defined as the preformed ripening substances.

The amount of these varies in the various organs and on the whole it may be said that the concentration is low, since the largest

¹ Determination of plasma on freshly collected blood (average of 11 animals)

² " " " " " " " (average of 9 animals)

³ " " " " " " " (average of 15 animals)

⁴ " " " " " " " (average of 5 animals)

The values given in parantheses are determinations on the freshly expressed juice.

The ripening indexes of the stored juice are the values immediately preceding them.

Table 2.

Ripening index for extracts of organs (upper values). (1 g extracted with 10 ml saline).

Ripening index for extracts of organs to which tyrosine has been added (0.1 cm³ 1 %) (lower values). Correction for effect of tyrosine 0.03.

The organs from ox and pig are derived from several different animals, the rest of the animal organs from the same animal.

Plasma ¹	1.00 —	0.92 —	0.89 0.92	0.82 0.84	0.80 —	0.71 0.74	0.57 0.59	0.48 0.51
Brain	0.08 0.12	0.07 0.10	0.07 0.14	0.04 0.07	0.06 0.09	0.06 0.07	0.05 0.05	0.03 0.05
Heart	0.22 0.47	0.20 0.38	0.21 0.42	0.17 0.38	0.17 0.34	0.15 0.31	0.13 0.25	0.11 0.22
Striated muscles	0.22 0.40	0.21 0.37	0.20 0.37	0.18 0.34	0.17 0.27	0.16 0.26	0.14 0.23	0.12 0.18
Lung	0.04 0.04	0.03 0.03	0.04 0.04	0.04 0.03	0.03 0.04	0.03 0.03	0.04 0.03	0.03 0.03
Kidney	0.04 0.03	0.04 0.04	0.04 0.04	0.04 0.04	0.03 0.04	0.03 0.02	0.03 0.03	0.02 0.03
Thymus	0.09 0.10	—	—	—	0.05 0.07	—	—	—
Spleen	0.19 0.30	0.18 0.28	0.17 0.27	0.17 0.46	0.15 0.25	0.14 0.21	0.13 0.16	0.11 0.13
Bone marrow ..	0.60 0.92	—	—	—	0.48 0.72	—	—	—
Liver	0.30 1.03	0.30 0.90	0.29 0.88	0.27 0.86	0.33 0.91	0.21 0.67	0.17 0.58	0.13 0.53
Stomach	see Abomasum	0.25 1.18	0.26 1.25	0.18 1.04	0.24 2.88	0.21 1.07	0.17 0.96	0.15 0.88
Tun. mucosa ventriculi					0.23 2.65			
Tun. muscular. ventriculi					0.13 0.58			
Cardia					0.22 2.04			
Fundus					0.18 1.91			
Pylorus					0.28 2.58			
Abomasum ..	0.29 1.88							
Rumen + reti- culum	0.06 0.10							
Psalterium	0.08 0.08							

¹ Plasma values determined in previous experiments. Plum (1943).

amount is found in the plasma, liver, spleen and bone-marrow; but taking the individual organs as a whole it is, nevertheless, not small amounts which are found in the large, e. g. in the muscles.

The rise in the activity by the addition of tyrosine to the extracts showed in what organs the thermolabile factor was found which has here been defined as »preformed ripening substance».

As far as all normal animals are concerned the plasma cannot be activated by tyrosine and so it contains exclusively fully ripened, not preformed maturing substances.

Some of the extracts derived from organs not taking any direct part in the formation of blood cells show no power of activation either.

The spleen and bone-marrow contain about $\frac{2}{3}$ of the total amount of maturing substances in fully ripened form, the rest is preformed. In the liver there is only about $\frac{1}{3}$ in the shape of fully ripened maturing substance. All these determinations were carried out with extracts made by extraction of 1 g dried organ with 10 cm³ saline.

The investigations on the stomach show that pure stomach extract only contains »fully ripened maturing substances» in amounts fairly similar to what is found in the organs that do not enter into the erythropoiesis. The experiments on the activation of the preformed maturing substances revealed, however, that the stomach contained considerably larger amounts of preformed maturing substance than any other organ, the effect of the substances being here increased from 5—10 times by adding tyrosine.

Investigations on conditions in the ox showed that a really substantial content of fully ripened as well as preformed maturing substances was only found in the abomasum, while the psalterium and the rumen + the reticulum only contained insignificant amounts. From these observations it must be inferred that the formation of the reticulocyte-ripening substances must be associated with that part of the stomach where the actual production of digestive enzymes takes place, while the psalterium, the reticulum and the rumen are only of mechanical significance for the digestion and allow certain bacterial decompositions.

The formation of the substances is thus apparently associated with the secretory of the stomach. In order to investigate this question the pig's stomach was divided a. m. Meulengracht (1935)

and here it was found that the largest amount of fully ripe maturing substances was present in the pylorus, the smallest in the fundus, but by the addition of tyrosine fairly similar activation was obtained.

By division of the stomach wall it was ascertained whether the formation of the substances was associated with tun. muscularis or tun. mucosa. It turned out that the amount of fully ripened substances was greatest corresponding to tun. mucosa, but here as the examination of the various parts of the stomach it was seen that the proportion of fully ripened and preformed substances was so to speak the same in the strata examined.

The question as to whether the stomach secreted the preformed substance was investigated on fasting gastric juice from human subjects.

The gastric juice recovered had a pH of about 2, but this was neutralised by $n/10$ NaOH to pH 7 before being used for the experiments.

The content of fully ripened substance in the gastric juice is rather small, but here too a considerable activation is seen upon the addition of tyrosine, so that substantial amounts of the preformed substance are also found in the human gastric juice (Table 3).

It then appears from the results obtained that there is a great difference between the ability of the different organs to produce reticulocyte-ripening substances, and that the power of forming

Table 3.

Analysis of fasting gastric juice from human subjects for its content of reticulocyte-ripening substances.

	pH	Pepsin	Dry matter	Ripening index for neutral gastric juice	
				cm ³ Gastric juice + 0.2 NaCl 0.9 %	cm ³ Gastric juice + 0.2 Tyrosine 1 % ₁₀₀
R. P. ♀, 28 years..	1 < pH < 2	6.24 mg%	—	0.11	0.55
J. H. ♂, 37 years	1 < pH < 2	—	0.38%	0.11	0.59
E. K. ♀, 27 years..	1 < pH < 2	—	0.46%	0.14	0.66
J. M. ♀, 25 years..	1 < pH < 2	—	0.39%	0.13	0.62
C. M. P. ♂, 29 years	1 < pH < 2	6.32 mg%	—	0.15	0.58

them depends on two factors, viz. 1) the ability to form preformed substance, and 2) the ability to couple the preformed substance with tyrosine or a related substance whereby the reticulocyte-ripening substance itself is obtained. It must be assumed that those organs in which the ratio between fully ripe and preformed maturing substance is the lowest are probably the organs in which the preformed substance, identical with the so far unknown thermolabile factor, is formed.

This is the case in the stomach where the ratio in question is $1/5$ — $1/10$, while the corresponding ratio for the organ next in succession, the liver, as already mentioned, in $1/3$.

These results would seem to indicate that the production of the preformed substances takes place in the stomach wall, from which they pass into the reticulo-endothelial system where the actual ripening, i. e. the linking with tyrosine or a tyrosine-like substance occurs, cf. Jacobsen & Plum (1943). This may take place directly from the stomach through the blood stream or by the preformed ripening substances being secreted through the glands of the stomach into the gastric juice and from there passing with the food into the intestinal canal where the resorption will then take place.

These investigations on the content of reticulocyte-ripening substances in the stomach and the localisation of the site of formation agree in a remarkable way with those of Castle concerning the formation of the »intrinsic factor».

Thus the importance of the stomach for the maintenance of the normal red blood picture, first stressed in Castle's investigations, has been revealed in one more domain.

On the chemical nature of the reticulocyte ripening substances in the stomach further investigations are made and this will be published collaborated with Dr. med. Erik Jacobsen.

Summary.

A report is given of the occurrence of fully ripe as well as preformed (tyrosine activable) reticulocyte-ripening substances in various animal organs. Of the fully ripe maturing substances the greatest amount is found in the plasma, liver, spleen and bone-marrow. Preformed (tyrosine activable) reticulocyte-ripening sub-

stances are not normally found in the plasma. In the spleen and bone-marrow about $\frac{1}{3}$ is tyrosine activable, in liver about $\frac{2}{3}$ and in the stomach $\frac{4}{5}$ — $\frac{9}{10}$. Of all the organs examined the stomach contains the largest amount of fully ripe + preformed maturing substances, determined on extracts of the various organs, made by extraction of 1 g dried with 10 cm³ saline.

Considerable amounts of ripening substances were found in the abomasum, i. e. the secretory part of the stomach of ruminants, none in the non-secretory parts of the stomach.

In pig's stomach the largest amount of fully ripe maturing substance was found in the pylorus: by division of the stomach wall the greater part was found corresponding to the tunica mucosa, while the proportion of fully ripe and preformed substance was fairly similar throughout the stomach.

In the fasting gastric juice of normal human subjects but little fully ripe maturing substance was found, but a considerable activation occurred upon the addition of tyrosine.

These results are interpreted to mean that the preformed ripening substances are formed in the stomach and in some way or other are carried to the reticulo-endothelial system where they are linked by tyrosine to the fully ripe maturing substances found in the plasma.

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Pain and Rectal Tenesmus after Injection of Adrenaline into the Colon.

By

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(Submitted for publication February 12, 1944).

Introduction.

In a previous work (1942) it was shown that administration of 2—3 mg adrenaline in 4—6 ml 35 % alcohol to patients suffering from ulcer in most cases gave rise to dyspeptic symptoms which as a rule closely resembled the patients' spontaneous attacks of cardialgia. The pain might become very intense irrespectively of whether there were hunger contractions at the same time or the stomach was entirely quiescent. Incidentally the adrenaline has an inhibitory effect on the motility of the stomach.

If the stomach was observed through a gastroscope simultaneously with the injection of some millilitres of an alcoholic solution of adrenaline, pale stripes could be observed corresponding to the area with which the adrenaline solution had been in contact. In other words, a limited anemia of the mucosa arose in connection with the subjective dyspeptic symptoms.

If adrenaline in 35 % alcohol was administered by mouth to healthy individuals in the same doses as to the ulcer patients (1943) a sensation of hunger might ensue; in some of them it would be stronger than they ever remembered to have felt it before. At the same time there was as a rule some suction in the epigastrium. In other healthy persons there only occurred dyspeptic symptoms

just as in the ulcer patients. The addition of nupercaine (percalinum »Ciba») to the adrenaline solution, which gave the solution a strong locally anesthetising effect, did not change the picture. The dyspepsia or hunger still set in and, as far as could be judged, with undiminished intensity. Owing to this experience of the effect of adrenaline on the gastric mucosa it seemed of interest to investigate the effect of its application to other parts of the digestive tract. As the place most easily accessible the sigmoid colon was chosen, or now and then the transverse colon, as an adrenaline solution could without difficulty be injected into this part of the digestive canal in patients on whom colostomy had been performed.

Material and Technique.

The investigations were made on 23 patients, on whom, as already mentioned, colostomy had been performed. 21 of the patients were suffering from rectal cancer and 2 from cancer of the colon. The diagnoses were in all cases verified by microscopy.

As far as could be done patients were selected who were as little as possible affected, psychically and physically, by their disease, and special weight was attached to the fact that the patients had had no pain, or no appreciable pain, during the last few weeks before the experiment.

The experiments were made in the morning without previous fasting. The adrenaline solution was injected into the colon by means of a syringe and a small rubber tube which could as a rule without difficulty be introduced into the intestine. Where nothing else is stated the injection was made in the oral crus of the exposed colon. In a couple of experiments where an adrenaline solution with nupercaine added had been used in the first injection the adrenaline solution was later injected alone in the anal part of the colon.

At first 1.5—3 ml 1^o/₁₀₀ adrenaline solution was used. Next 1 ml of a solution of $\frac{1}{3}$ to $\frac{1}{2}$ % was injected. As the injection of adrenaline in an alcoholic solution caused pronounced resorptive symptoms (Experiment 11), it became necessary to confine oneself to the use of aqueous solutions which only in some few cases gave rise to resorptive symptoms and if so, quite mild ones.

In order further to elucidate the mechanism of the genesis of the pain 2 % nupercaine was in several cases added to the adrenaline solution.

Experiment 1.

E. C. J. Man, aged 27.

Diagnosis: Rectal cancer.

No pain or discomfort in the abdomen after colostomy performed 30 days previously. The patient felt well at the beginning of the experiment.

At 10.48 o'clock injection of 1.5 ml 1⁰/₁₀₀ adrenaline solution about 3 cm inside the colostomy opening.¹ 12 minutes later a moderate sensation of flatulence in the preternatural anus; the sensation subsided after about 20 minutes.

At 11.20 injection of 2 ml 1⁰/₁₀₀ adrenaline solution about 5 cm inside the colostomy opening. 9 minutes later a sensation of flatulence as after the first injection. From 11.43 to 12.05 rather troublesome colic-like pains diffusely in the left iliac fossa and round the umbilicus. After that the symptoms gradually subsided. At 12.45 just after the patient had had dinner acute twinges occurred diffusely throughout the abdomen, so that the patient had to lie down on his bed; also strong nausea. In the course of three quarters-of-an-hour to one hour the worst pain was over, but not until 16 o'clock did the patient feel perfectly well again.

No symptoms of resorptive adrenaline effect.

The experiment showed that severe colic-like pains set in diffusely in the abdomen after injection of an adrenaline solution into the sigmoid colon.

Experiment 2.

E. C. B. M. Woman, aged 51.

Diagnosis: Rectal cancer. Metastases to the column.

Colostomy performed 12 months previously. Often pains in the shoulder and lumbar regions, but never in the abdomen. No feeling of discomfort whatever at the beginning of the experiment.

At 9.46 o'clock injection of 3 ml 1⁰/₁₀₀ adrenaline solution about 7 cm inside the colostomy opening. No subsequent symptoms.

At 10.13 injection of 1 ml ½ % adrenaline solution about 10 cm inside the colostomy opening. 7 minutes later rectal tenesmi set in. From 10.25 to 11.00 there were pains and twinges and a sensation of flatulence right across the abdomen. There were likewise severe rectal tenesmi resulting in evacuation by the natural way. The pain was at times very unpleasant.

At 11.00 administration of 2 Eukodal tabloids after which the symptoms subsided in the course of 15—20 minutes.

No symptoms of resorptive adrenaline effect.

¹ Where nothing else is stated, the injections were always made in the oral crus of the colon.

The experiment showed that severe colic-like pains diffusely throughout the abdomen as well as rectal tenesmi came on after injection of an adrenaline solution in the sigmoid colon.

Experiment 3.

A. E. S. Woman, aged 64.

Diagnosis: Rectal cancer.

Colostomy performed 25 days previously. During the last 5 weeks often rumbling and a sensation of discomfort increasing to pain in the lower part of the abdomen, principally in the night. No pain or discomfort at the beginning of the experiment.

At 9.13 injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the colostomy opening. After 7 minutes incipient oppression downwards in the abdomen as well as rectal tenesmi. In the course of the next 20 minutes increasing, at times very unpleasant, oppression downwards in the abdomen and strong rectal tenesmi. At 9.37 subcutaneous administration of 0.3 ml 1 $\frac{1}{100}$ histamine to relieve the pain. Pain and tenesmi then gradually subsided during the next 20 minutes.

The symptoms exactly resembled those the patient might have in the night.

No symptoms of resorptive adrenaline effect.

The experiment showed that severe oppression downwards in the abdomen as well as very troublesome rectal tenesmi set in after injection of an adrenaline solution in the sigmoid colon.

Experiment 4.

K. I. A. B. Woman, aged 61.

Diagnosis: Cancer of the colon.

Upwards of 4 months prior to the experiment excision of part of the colon as well as an adjoining piece of the small intestine. One month prior to the experiment an operation was performed for the purpose of closing the colostomy. When the experiment was made only a small colostomy opening was left. No pain or sensation of discomfort in the abdomen for the last four months apart from the fact that there might be a few twinges upon evacuation when aperients had been administered. There was now evacuation by the natural way every 2nd day by means of an aperient. At the beginning of the experiment the patient felt well.

At 9.26 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the intestine through the remaining small opening. From 9.40 to 9.58 first a somewhat unpleasant sensation of flatulence across the lower part of the epigastrium and a little later moderate diffuse pains in the abdomen as after an aperient. The symptoms then gradually subsided.

At 10.31 o'clock injection of $\frac{1}{2}$ ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the intestine. About 12 o'clock rather severe pain came on diffusely

in the abdomen and straining to evacuate the bowels though nothing came. The symptoms subsided in the course of $\frac{1}{2}$ hour. After this no abdominal symptoms.

No signs of resorptive adrenaline poisoning.

The experiment showed that a sensation of flatulence, a necessity to evacuate the bowels and rather severe pain diffusely in the abdomen came on after injection of an adrenaline solution in the transverse colon.

Experiment 5.

A. L. V. H. Male, aged 55.

Diagnosis: Rectal cancer.

Two months prior to the experiment excision of the rectum, after transversostomy had been performed 4 weeks previously. No pain or discomfort in the abdomen for a long time. Felt well at the beginning of the experiment.

At 9.45 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 5 cm inside the colostomy opening. Part of the fluid injected, perhaps half, ran out again, which at once made the mucous membrane of the preternatural anus pale. At the same time there came a slight sensation of smarting which was felt profoundly to the colostomy.

From 9.47 to 10.57 o'clock a very unpleasant sensation of tension and flatulence principally in the right side of the abdomen, increasing in periods to fairly acute griping pains resembling the sensation before diarrhea. At 10.50 subcutaneous administration of 0.3 mg histamine. Not until 11.30 o'clock were the symptoms almost relieved.

No signs of resorptive adrenaline effect.

The experiment showed that fairly pronounced sensations of flatulence and griping pains came on after injection of an adrenaline solution into the transverse colon.

Experiment 6.

L. S. Female, aged 61.

Diagnosis: Rectal cancer.

Colostomy performed 4 weeks previously. No pain or discomfort in the abdomen for the last fortnight. On the other hand, there were now and then pains in the anal region. The patient felt well at the beginning of the experiment.

At 9.30 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the colostomy opening. After 8—10 minutes increasing sensation of flatulence, weight and uneasiness in the abdomen principally localised to the left iliac fossa. The sensations of uneasiness increased somewhat for the next 25 minutes, whereupon they gradually subsided again. There was

no actual pain. — Though the patient several times had the sensation that feces were passing through the colostomy, there was no evacuation during the whole of the experiment.

The symptoms following the injection of adrenaline were something quite unusual for the patient.

No signs of resorptive adrenaline effect.

The experiment showed that an unpleasant sensation of flatulence, weight and uneasiness in the abdomen as well as a sensation that feces were being passed came on after injection of an adrenaline solution in the sigmoid colon.

Experiment 7.

C. T. J. Male, aged 56.

Diagnosis: Rectal cancer.

Colostomy performed 3 weeks previously. No appreciable feeling of discomfort in the abdomen after the colostomy. For the last 8 days no discomfort of any kind in the abdomen. After examination under anesthesia 2 days before the experiment, moderate pain persisted round the anal region, often accompanied by rectal tenesmi.

At the beginning of the experiment there were slight rectal tenesmi and moderate pain around the anus.

At 9.13 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the colostomy opening. After 4 minutes a sensation of tension and flatulence came on in the right iliac fossa, as when the bowels have not acted for some days. The sensation increased somewhat in intensity and gradually moved over towards the left side. After 15 minutes the sensation of discomfort again subsided and had practically disappeared by 9.48 o'cl.

At 9.52 injection once more of 1 ml $\frac{1}{3}$ % adrenaline solution about 8 cm inside the colostomy opening. 3 minutes later an unpleasant sensation of tension and flatulence around the umbilicus and in the right iliac fossa suddenly came on. The sensation, which exactly resembled the symptoms after the first injection, in the course of 5 minutes passed over towards the left iliac fossa. At 10.05 o'cl. the sensation of discomfort was rapidly decreasing and a few minutes later it had quite disappeared.

The symptoms from the anal region persisted quite unaltered during the experiment. There was no sign of any resorptive adrenaline effect.

The experiment showed that an unpleasant sensation of tension and flatulence came on few minutes after the injection of an adrenaline solution in the sigmoid colon. When all sensations of discomfort had disappeared, adrenaline was again injected, after which the same symptoms recurred.

Experiment 8.

M. N. J. Female, aged 50.

Diagnosis: Rectal cancer.

5 weeks prior to the experiment excision of the rectum and the lower part of the sigmoid colon, colostomy having been performed 8 weeks previously. No pain or sensations of discomfort in the abdomen for the last fortnight. The patient felt well at the beginning of the experiment.

At 9.28 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the colostomy opening. 9 minutes later griping pains in the stomach came on and a feeling as if the bowels were to act. The pains which increased in intensity and were at times troublesome were localised to the area round the umbilicus. They persisted fairly unchanged for about 20 minutes whereupon they gradually subsided. Not until 10.50 o'clock had the feeling of discomfort practically ceased.

During the experiment copious stools were evacuated. No sign of resorptive adrenaline effect.

The experiment showed that rather severe pain round the umbilicus came on following injection of an adrenaline solution into the sigmoid colon.

Experiment 9.

B. T. Woman aged 44.

Diagnosis: Rectal cancer.

Colostomy performed 19 days previously. No pain or sensations of discomfort in the abdomen for the last 4 days. The last few days there was now and then some oppression by the anus as well as slight rectal tenesmi. These symptoms were also present at the beginning of the experiment, while there were no sensations of discomfort at all in the abdomen.

At 9.45 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution to which had been added 2 % nupercaine about 10 cm inside the colostomy opening. Immediately after some of the injected fluid ran out again which caused paleness of the preternatural anus. At the same time a small quantity of feces was evacuated. After 3—4 minutes, increasing pain around the umbilicus with working and rumbling in the abdomen and a feeling as if the bowels were going to act. This did not happen at that time, however, but not until a quarter-of-an-hour later. The pain increased during the next 5 minutes; the patient perspired and turned alternately red and pale. At 10.06 the sensations of discomfort had abated a good deal, but a few minutes later a feeling of distension and weight in the lower part of the abdomen came on which during the next minutes was superseded by regular pain downwards in the epigastrium. In the course of 5—7 minutes the pain had disappeared but returned again at 10.31 o'clock. It now persisted almost unchanged for over 10 minutes, was diffusely dispersed all over the abdomen and was very disagreeable. There was also slight nausea. Then the pain subsided and had almost disappeared at 11.00 o'clock.

No signs of any resorptive adrenaline effect.

The experiment showed that colic-like pains came on diffusely in the abdomen after injection of a solution of adrenaline and nupercaine in the sigmoid colon.

Experiment 10.

H. P. P. B. Male, aged 62.

Diagnosis: Rectal cancer.

Colostomy performed 18 days previously. Since then some smarting round the colostomy opening, also periodically, last the day before the experiment, rather severe pains in the left iliac fossa.

Immediately prior to the experiment there was some working in the abdomen but no pain.

At 9.35 o'clock injection of 1 ml $\frac{1}{3}$ % adrenaline solution to which was added 2 % nupercaine about 11 cm inside the colostomy opening. A little of the fluid injected at once oozed out and caused pallor of the mucous membrane of the preternatural anus; at the same time some feces were passed. Immediately after the injection strong smarting and burning came on, localised to the exposed intestine. The pains which were very unpleasant, came on in fits and persisted for the next 20 minutes. They radiated from the colostomy partly into the left side and partly downwards. The patient also felt a little nausea which otherwise he very rarely suffered from. At about 10.14 o'clock the symptoms had subsided and the patient felt quite well again.

No sign of any resorptive adrenaline effect.

The experiment showed that a strong smarting and burning came on in the sigmoid colon following injection of a solution of adrenaline and nupercaine into it.

Experiment 11.

A. J. S. Woman, aged 61.

Diagnosis: Rectal cancer.

Colostomy performed well over 4 weeks previously. No pain or discomfort in the abdomen since the operation. The patient was well at the beginning of the experiment.

At 9.43 o'clock injection of 1 ml $\frac{1}{2}$ % adrenaline solution about 10 cm inside the colostomy opening. After 9 minutes some rectal tenesmi set in and 7 minutes later a peculiar sensation, unusual for the patient, of flatulence in both iliac fossae which, however, quickly disappeared again.

At 10.17 injection of 1 ml $\frac{1}{3}$ % adrenaline solution about 10 cm inside the intestine, without any symptoms from the abdomen nor any sign of resorptive adrenaline effect.

At 10.53 o'clock injection of 1 ml of a solution of $\frac{1}{2}$ % adrenaline in 35 % alcohol, likewise about 10 cm inside the intestine. Few minutes after palpitation set in, and in the course of 20 minutes the pulse frequency in-

creased from 80—84 to 124. Gradually the patient also turned somewhat pale and her hands began to tremble. Several nitroglycerin tabloids were administered in the course of the next hour. But as late as 12.35 o'cl. the pulse was 110 and not until about 14 o'cl. had the palpitation subsided. The patient vomited twice, at 12.25 and a little past 13 o'cl. respectively; at no time was there any pain in the abdomen.

The experiment showed that a brief sensation of flatulence and slight rectal tenesmi set in after injection of an aqueous solution of adrenaline into the sigmoid colon while a rather strong and protracted resorptive adrenaline effect was observed after injection of an alcoholic solution of adrenaline into the sigmoid colon, but no pain.

Experiment 12.

V. E. F. Male, aged 66.

Diagnosis: Rectal cancer.

Colostomy performed well over 3 weeks earlier. No pain or discomfort in the abdomen since the operation. At the beginning of the experiment the patient felt well.

At 9.45 o'cl. injection of 1 ml $\frac{1}{3}$ % adrenaline solution to which had been added 2 % nupercaine about 12 cm inside the colostomy opening. After 5 minutes, strong rectal tenesmi of few minutes' duration set in. No discomfort in the abdomen.

At 10.20 injection of 1 ml $\frac{1}{3}$ % adrenaline solution in the anal part of the sigmoid colon; the solution being distributed with $\frac{1}{3}$ ml respectively 12, 10, and 8 cm from the colostomy opening. No discomfort and no symptoms of resorptive adrenaline effect appeared.

The experiment showed that brief rectal tenesmi set in after injection of an adrenaline solution, to which nupercaine had been added, in the oral part of the sigmoid colon. Injection of an adrenaline solution alone in the distal part of the sigmoid colon elicited no response.

Experiment 13.

A. B. S. N. Woman, aged 56.

Diagnosis: Rectal cancer.

10 months prior to the experiment excision of the rectum after transversostomy 5 weeks previously. No pain or discomfort in the abdomen since the operation. The patient felt well at the beginning of the experiment.

At 10.27 o'cl. injection of 1 ml $\frac{1}{3}$ % adrenaline solution 10 cm inside the colostomy opening. 7 minutes later there was a feeling of hotness in the skin around the umbilicus over an area of about 25×12 cm. Appa-

rently there was no change of colour or temperature. The sensation of hotness, which was something quite unusual for the patient, was pronounced for the first 20 minutes, but with varying intensity. Then it gradually subsided and disappeared entirely at 11.05 o'clock.

At 11.05 o'clock, injection of 1 ml $\frac{1}{3}$ % adrenaline solution to which had been added 2 % nupercaine well over 12 cm inside the colostomy opening. After 5 minutes, incipient sensation of hotness with the same localisation as the first time; it persisted unchanged for a couple of minutes, but was scarcely as intense as after the first injection. Then the feeling of hotness gradually subsided and had quite disappeared by 11.23 o'clock.

No pain or discomfort in the abdomen nor any sign of a resorptive adrenaline effect.

The experiment showed that a pronounced sensation of hotness in the skin around the umbilicus set in after injection of an adrenaline solution to which nupercaine had been added. Apparently there was no change in the colour or temperature of the skin.

In 6 experiments besides those reported here 1 ml $\frac{1}{3}$ % or $\frac{1}{2}$ % adrenaline solution was injected 2 or 3 times at about half an hour's interval, that is to say, 6—13 mg adrenaline in all, about 10 cm inside the sigmoid colon, without eliciting any response either in the form of sensations of discomfort in the abdomen, rectal tenesmi or signs of a resorptive adrenaline effect. In one of these cases 2 % nupercaine had been added to the adrenaline solution, in another instance 1 ml $\frac{1}{3}$ % adrenaline solution with 2 % nupercaine was first injected in the oral part of the intestine and 40 minutes later 1 ml $\frac{1}{3}$ % adrenaline solution alone in the anal crus.

In another 4 patients 1 ml $\frac{1}{3}$ % adrenaline solution to which 2 % nupercaine had been added was injected, without causing discomfort in the abdomen or rectal tenesmi. On the other hand, mild symptoms of resorptive adrenaline effect came on, in the shape of a rise in the frequency of the pulse, and in a couple of cases also palpitation, pallor, and tremor.

Experimental results.

The experiments showed that in 10 of the 23 patients more or less pronounced sensations of discomfort came on from some few to 14 minutes after the injection of adrenaline. The symptoms were an unpleasant sensation of tension, flatulence, and coliclike

pains diffusely in the abdomen and of more or less painful rectal tenesmi. In several cases the symptoms were reminiscent of the twinges that may occur after the taking of aperients or before diarrhea. There was often a strong feeling that the bowels were going to act, but as a rule no feces came. The symptoms came on after a single injection of adrenaline.

In 2 cases (experiments 11 and 12) only slight rectal tenesmi occurred, and in a single experiment (No. 13) a peculiar sensation of hotness around the umbilicus.

In the remaining 10 experiments it proved impossible to elicit sensations of discomfort in the abdomen. In some of these patients there were mild symptoms of a resorptive adrenaline effect.

The typical sensations of discomfort might appear whether or not nupercaine had been added to the adrenaline solution (experiments 9 and 10). But even if not very pronounced or no symptoms occurred in several cases after injection of an adrenaline-nupercaine solution, the cause of this can hardly have been the admixture of nupercaine, in so far as repeated administration of adrenaline alone in the anal crus of the colon in two of these cases elicited no response.

Comments.

While administration of some few milligrammes of adrenaline in an alcoholic solution caused cardialgia and in some cases a sensation of hunger in the great majority of the subjects examined, healthy individuals or ulcer patients (1943 and 1942), the effect of the somewhat larger doses of adrenaline in an aqueous solution applied to the colon was not nearly as certain, seeing that only pronounced sensations of discomfort occurred in barely half of the patients.

On the other hand, the pain response occurred too often for it to have come on accidentally, not least if we consider that the majority of patients in question had had no feelings of discomfort in the abdomen at least for a fortnight prior to the experiment. To this must be added that the symptoms after entirely subsiding could now and then be elicited again by renewed adrenaline injection (experiments 4 and 7).

The capricious effect was presumably due partly to the fact that the stimulus only issued from a comparatively very small

area of the mucosa and partly to a differing sensitivity in the patients, similarly as an appendicitis may in some cases develop up to the point of rupture of the appendix without the patient having felt pain. A defective contact with the mucosa owing to mixing of the adrenaline solution with the content of the intestine may, however, also have played its part.

After oral administration of adrenaline the pains were always localised to the cardia and the upper part of the abdomen, so they might well be conceived to have issued exclusively from those areas of the stomach wall which were affected by the adrenaline. After injection of adrenaline into the colon pain might likewise occur corresponding to the site of application, for instance in the form of smarting of the exposed intestine itself, while the cozing out of a little of the adrenaline solution had made the mucosa anemic (experiment 10). But in most cases the pains occurred far from the site of application, for instance in the right side or diffusely throughout the abdomen.

It must be regarded as highly improbable that the injected one millilitre of adrenaline solution can have affected more than a quite limited area of the mucosa near the site of application: the pains must therefore be assumed to be principally conditioned by reflexes. This must at any rate be true of the not rarely occurring rectal tenesmi which set in after injection of adrenaline into the oral part of the colon (experiments 2, 3, 11, and 12).

What changes led to the onset of pain could not be gathered from the experiments. The sensation of working in the intestine which now and then accompanied the pain may perhaps indicate that spastic contractions were elicited by reflex effects.

As is well known, it does not cause pain to cut, burn, or pinch an intestine. On the other hand, pain will be elicited by vigorous contractions of the smooth muscles, volvulus conditions, incarceration of hernias and great distension, and as shown in this paper, sometimes as a result of the action of adrenaline on the mucosa. A closure of the capillary areas corresponding to the contracted, the distended, the incarcerated part of the intestine, or the part affected by adrenaline, must be a common factor in these different conditions, and it would not be unreasonable to see in this the primary cause of the pain.

The mechanism in the release of pain has been thoroughly

discussed in previous papers to which the reader is referred (1942, 1943, and 1944). It was conjectured that the vasoconstriction in the mucosa, by retroaction on the arteries carrying blood to the contracted capillaries and arterioles, caused a dilatation of the arteries concerned and perhaps an increase in amplitude by which peri-arterial pain-percipient nerves were irritated.

The correctness of this hypothesis has been confirmed by measurements of the arteries in the mesentery of the stomach of rats following injection of an adrenaline solution into the lumen of the stomach close to the measured artery. A considerable dilatation of the measured arteries was then observed in by far the majority of cases (1944).

The local pain in connection with injection of adrenaline into the colon would then be due to dilatation of the arteries in the tissue under the anemic area of the mucosa, while pain and tenesmi far from the site of application might be conceived to have arisen by reflex action with, for instance, spasms or distensions, and a capillary occlusion and subsequent dilatation of the arteries caused thereby as a pain-eliciting factor.

Summary.

In previous works it was shown that oral administration of adrenaline would often cause cardialgia and a feeling of hunger.

In order to ascertain the effect of the application of adrenaline in other parts of the digestive tract aqueous solutions, as a rule consisting of 1 ml $\frac{1}{2}$ — $\frac{1}{3}$ % adrenaline, were injected into the colon in 23 patients with colostomy.

In 10 of these pronounced discomfort in the abdomen came on, in the shape of colic-like pains, an unpleasant sensation of flatulence or painful rectal tenesmi. The pain would come on whether or not 2 % nupercaine had been added to the adrenaline solution.

The remaining 13 subjects on the whole remained unaffected, apart from the fact that brief rectal tenesmi occurred in two cases, while in some instances mild symptoms of a resorptive adrenaline effect occurred.

In accordance with a hypothesis previously advanced it was supposed that the pain at the site of application was caused by a dilatation of arteries in the tissue under the anemic area of the

mucosa, while pain and tenesmi far from the site of application were supposed to have arisen by reflex action with, for instance, spasms or distensions and a resulting capillary occlusion leading to arterial dilatation in the underlying tissue as a paineliciting cause.

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On the combination, diabetes mellitus and acute hepatitis.

By

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(Submitted for publication January 31, 1944).

The anatomy and physiology of the liver in diabetes mellitus have been discussed in the literature on several occasions during the last few decades. Rabinowitch (1936) pointed out that as early as 1748 it was discovered that diabetics may show fatty degeneration of the liver. Dalous, Fabre and Valdiguie (1936) were able to demonstrate degenerative fatty infiltration and deposits of a melanin-like substance in the cells of the liver in two patients who died in diabetic coma. The connective tissue of the liver in these cases exhibited hyperplasia of toxic, not inflammatory origin. van Noorden, on the other hand, was of the opinion that anatomic injury to the liver in diabetes mellitus is rare. Weichmann made the same observation. Hepatic enlargement in diabetes was mentioned by van Noorden and Isaac and by Umber. Hanssen (1936) found hepatic enlargement in 12 out of 44 diabetic patients under 20 years old, but in only one out of 231 diabetics over 20 years. Court (1938) published two cases of diabetes in children combined with pronounced hepatomegalia.

Krarup and Iversen (1940) took biopsy specimens of the liver in two cases of hepatic enlargement in diabetes mellitus. Histologically the enlargement was found to be due mainly to edema in the liver cells and only in part to diffuse fatty infiltration. Drag-

stedt suggested two possible causes of enlargement of the liver in diabetes mellitus: firstly, a lack of pancreatic hormone (other than insulin); and secondly, faulty treatment of the disease of diabetes itself (possibly undiagnosed acidosis).

Stockinger and Wenzel (1938) often found considerable hepatic enlargement in cases of combined diabetes and hepatitis. For example, the liver was palpable two to four fingers below the costal arch in 16 out of 24 cases. There was also pressure tenderness over the liver in some of these cases.

Jacobi (1936) carried out glucose tolerance tests on patients with acute hepatitis. The curves he secured were either relatively level or rose sharply. The normal value was reached within two hours. Pachman (1940) was unable to show any correlation between the degree of jaundice and the type of glucose tolerance curve.

Rabinowitch (1926) found hyperbilirubinemia in 24.1 per cent of 130 cases of diabetes. Later (1936) he published the results of 3000 analyses of bilirubin in the blood and 3000 analyses of urobilinogen in the urine of diabetics. All cases of definite or suspected jaundice were eliminated from the investigation. Hyperbilirubinemia was found in 27.4 per cent of the cases. Hyperurobilinogenuria could be demonstrated in 27.5 per cent of 3000 urine analyses, in which the normal content of urobilinogen was considered to be the amount of urobilinogen which could be found in a dilution of the urine up to 1:20. Rathery, Polydorides and de Traverse (1939) in 590 analyses of the urine of 325 diabetics found a distinct increase in the bilirubin content in 50.4 per cent. Weichmann on the other hand secured a positive urobilinogen reaction in only 2.4 per cent of his diabetes patients.

The data as to the incidence of diabetes mellitus combined with clinically established acute hepatitis varies. Flaum, Malmros and Persson (1926) published a report on an epidemic of hepatitis in Lund in 1923 involving 34 cases, 28 of which were combined with diabetes mellitus. Steinitz (1931) observed an accumulation of hepatitis in diabetes patients. The same worker later (1932) published a comparative study of 334 diabetes cases, among which there were 19 cases of hepatitis. Stockinger and Wenzel (1938) also observed diabetes combined with hepatitis relatively frequently. The incidence of this combination was shown to vary between 0.7 and 8.0 per cent in a table giving the annual statistics

for the years 1933 to 1936. In a few cases Stockinger and Wenzel observed hepatitis in diabetes patients treated with synthaline. Singer, Naunyn, van Noorden, Umber, and Lichtwitz did not observe any increase in the incidence of hepatitis among diabetes patients.

According to Falta (1935) parenchymatous jaundice is not more common among diabetics than non-diabetics. He considered that the common combination of diabetes and other hepatic diseases is due, for example, to the fact that an infection may easily attack both the pancreas and the liver, or that faulty habits (overeating, alcoholism) have a deleterious effect on these two organs. Ducas and Uhry (1939) seldom observed hepatitis in diabetics.

Steinitz found the diabetes-hepatitis combination more often in women than men, but concluded that the degree of severity of diabetes, like the age of the patient, was of no significance to hepatitis. Nor could Stockinger and Wenzel observe any disposition caused by age. The two diseases more often appear concurrently in the winter than the rest of the year, according to Steinitz, but Stockinger and Wenzel observed no seasonal variation. In the opinion of the two latter workers, the period elapsing between the onset of diabetes and the appearance of hepatitis varies greatly. The duration of the jaundice in their cases ranged between two and ninety days.

Flaum, Malmros and Persson considered the cause of the high rate of hepatitis in diabetics to be an infection incurred in connection with the taking of blood samples with Francke's needle for the determination of the sugar content of the blood. This manner of infection was doubted by Steinitz and rejected by Stockinger and Wenzel. The two last-mentioned workers stated that they were unable to advance a satisfactory explanation of the common combination of the two diseases. Some of the patients affected with both complaints in their material had been treated with insulin, others had not. Medicamentous intoxication could be ruled out in all the cases. The Wassermann reaction was invariably negative. There was no reason to suspect typhoid or paratyphoid fever. Duodenal probing revealed nothing abnormal either microscopically or bacteriologically. The diabetes patients who also exhibited hepatitis originated in different parts of the district served by the hospital. Direct infection was therefore out of the question. Stoc-

kinger and Wenzel pointed out that the diabetes-hepatitis combination did not occur during a general epidemic of hepatitis. They attributed the cause of the combination in the patients treated with insulin to a temporary deterioration in the quality of the insulin used.

Selander (1942) published an account of 960 cases of hepatitis observed in Gothenburg, Sweden, during the years 1924 to 1938. Direct contact with a hospital, either as an in- or out-patient had occurred in 274 cases. All these 274 patients had been submitted to the hypodermic needle in some form or another — vaccination, injection, blood tests, etc. The time which elapsed between the onset of the disease and contact with a hospital varied greatly, amounting to an average of two to three months. Selander considered that an acute hepatitis with a long period of incubation frequently results from direct inoculation in the taking of a blood sample, etc. The long incubation distinguishes this inoculation hepatitis from the usual epidemic hepatitis. Selander also showed that inoculation hepatitis unlike epidemic hepatitis exhibited no seasonal variation and did not spread to the people in contact with the patient. Selander concluded that if the virus is the same as in epidemic hepatitis, inoculation with the virus is followed by a longer incubation than the usual manner of infection, and the disease when incurred by inoculation does not spread in the usual way.

The Writer's Material.

During recent years, particularly in 1942, the diabetes-hepatitis combination has often been observed at the Medical Department of Centrallasarettet in Västerås. The reason for this increase in frequency of hepatitis in diabetic patients is unknown. A possible solution is that during recent years ordinary insulin in an increasing number of patients has been replaced by protamine-zinc insulin. A long series of daily injections of a solution containing zinc might conceivably have an injurious effect on the parenchyma of the liver, for example. At the suggestion of Dr. A. Bjure, I therefore made this possibility the basis for an investigation on a series of diabetic patients.

The investigation covers all the diabetic patients hospitalized at Centrallasarettet in Västerås or treated in the out-patient

department of that hospital during the year 1942. There were altogether 77 men and 116 women. Hepatitis was found in 9 of the men and 16 of the women, or in 11.7 per cent and 13.8 per cent, respectively. Acute hepatitis had been present in 25 or 12.95 per cent of all the diabetic patients, and of these 25 cases 18 occurred in 1942. The frequency of acute hepatitis among the diabetic patients under observation during 1942 thus amounted to 9.33 per cent. In 1942, 21 men and 11 women were treated at this hospital for acute hepatitis without diabetes. In addition, there were a small number of hepatitis cases treated in the out-patient department. Diabetes patients who contracted hepatitis can be assumed to have sought hospital care to a much greater extent than hepatitis patients who were otherwise healthy. Even with this reservation, however, it seems undeniable that diabetic patients contracted acute hepatitis much more often than other people. If this were not the case, at least 9 per cent of the whole population of the district served by the hospital would have contracted acute hepatitis.

Steinitz observed the diabetes-hepatitis combination more often in women than men. This was also true of my material. Of the 25 patients with diabetes who incurred hepatitis, 16 or 64 per cent were women. In a control series of 32 cases of acute hepatitis without diabetes, 34.4 per cent were women. However, the difference between the incidence of women with hepatitis and diabetes and of women with hepatitis alone amounted to only 29.6 ± 12.76 per cent. Hence no statistically established conclusion can be drawn regarding the sex incidence of diabetes and hepatitis on the one hand and of hepatitis only on the other hand.

The average age of diabetic patients at the onset of hepatitis was 44.2 years (range of variation 7 to 67 years). For non-diabetics who contracted hepatitis, the average age was 28.8 years (range of variation, 9 to 70 years). The difference in the ages of diabetics and non-diabetics at the onset of hepatitis was numerical only. That the average age of the diabetics at the onset of hepatitis was higher than the non-diabetics is connected with the fact that the average age of all the diabetics included in the investigation for the year under discussion was approximately 45 years. Thus a selection of relatively old patients was involved.

Jaundice apparently remained longer in the diabetic patients

than in patients with hepatitis only. The duration of the disease was calculated from the day mentioned in the case history when the patient first showed distinct signs of jaundice (icteric discoloration of the sclerae, the skin or the urine) until the Meulengracht icteric index had dropped to at least 12 (for practical reasons the patient's condition could not be checked longer with sufficiently frequent tests). The average duration of jaundice in diabetic patients was 8.3 weeks (range of variation, 2 to 17 weeks) and in non-diabetics 5.3 weeks (range of variation, $\frac{1}{2}$ to 16 weeks). Statistical calculation yields a difference here of 3.0 ± 4.79 weeks, or a numerical difference only.

The maximal strength of jaundice, i. e. the highest Meulengracht icteric index, averaged 71 with a range of variation of 17 to 180 in patients with diabetes and hepatitis and 67 with a range of variation of 13 to 240 in patients with hepatitis only.

In the patients with diabetes and hepatitis the curves for the Meulengracht icteric index and for the strength of the urobilin and Hammarsten tests of the urine showed no relationship with the occurrence of glycosuria or acidosis, with the height of the blood sugar value or with the duration of the hepatitis. Nor was there any definite connection between the appearance of these curves and the period of time during which the patient received protamine-zinc insulin. On the whole the curves for patients with diabetes and hepatitis resembled those for patients with hepatitis only, except that the curves in the former group were often more elongated than the latter. In both groups a negative response to the Hammarsten test was practically always immediately followed by a distinct and definitive reduction of the Meulengracht icteric index to normal. With a normal or nearly normal Meulengracht value positive reactions to the urobilin test were sometimes secured. Nor in this respect was there any difference between patients with diabetes and hepatitis and patients with hepatitis only.

Sublimate tests of the stools were made on 15 patients with diabetes and hepatitis and 25 patients with hepatitis only. These tests elicited positive reactions in 13 and 21 cases, respectively, or to about the same extent in both groups.

The liver was palpable in 11 out of 22 patients with diabetes and hepatitis. In one of these the liver was scarcely palpable; in 8 of them it was enlarged to one to two fingers below the costal arch;

and in 2 it was enlarged more than two fingers below the costal arch. Among 31 cases of hepatitis, the liver was palpable in only 7. In 3 of these cases the liver was scarcely palpable and in 4 it reached to one to two fingers below the costal arch. It should be added, however, that diabetes in itself often leads to palpable enlargement of the liver.

The white blood count at the time of hospitalization of the patients is shown in the following table:

No. of leukocytes	2000— 4000	4000— 6000	6000— 8000	8000— 10000	> 10000
Diabetes + hepatitis..	5 cases	6 cases	5 cases	5 cases	—
Hepatitis	2 »	16 »	11 »	1 case	2 cases

Six of the cases of hepatitis only covered by the investigation were submitted to glucose tolerance tests. In two of them normal tolerance curves were secured, while in the remaining four the curves showed suggested elongation so that the normal blood sugar value was not reached until after 2 ½ to 3 hours.

A study was made of the whole series of diabetes patients to determine whether the height of the blood sugar value or the presence of acidosis, glycosuria or albuminuria caused a disposition toward hepatitis. It was also attempted to discover whether the period of time during which the patient received Vitrum insulin or protamine-zinc insulin had any effect on the incidence of hepatitis. In connection with the investigation to find out what part, if any, was played by the blood sugar in hepatitis, an average, fasting blood sugar value was estimated for each patient for the period during which the blood sugar was followed. The mean value was then calculated on the basis of these approximate averages. The mean figure for blood sugar secured in this way was 0.20 g per cent for patients with diabetes only and 0.21 g per cent for patients with diabetes and hepatitis.

With regard to acidosis, glycosuria, and albuminuria, it was attempted to determine for the years during which the patients were under observation at this hospital whether these conditions were present constantly, frequently, or occasionally, or whether they were lacking as a rule or invariably. The figures for incidence

secured in this way have been graded from 5 to 1, yielding the following comparative figures for acidosis, albuminuria and glycosuria:

	Acidosis	Glycosuria	Albuminuria
Diabetes + hepatitis	2.1	4.0	1.6
Diabetes	1.8	3.9	1.4

One is undoubtedly not justified in drawing any conclusion from these small differences, particularly since the figures are only approximations. In addition, it was not possible to find any effect on the incidence of hepatitis resulting from insulin treatment of diabetics. In the group with diabetes only, 12.5 per cent of the cases had not been treated with insulin, while in the group diabetes and hepatitis, 12 per cent had not received insulin before the onset of hepatitis. As revealed in the following table, the period of time during which treatment with Vitrum insulin or protamine-zinc insulin, possibly combined with Vitrum insulin, was given was approximately the same for patients with diabetes only and for patients with both diabetes and hepatitis. In this respect, therefore, the two groups showed statistical identity.

	Diabetes	Diabetes + hepatitis
Average no. years treated with Vitrum insulin	6.7 yrs.	5.5 yrs.
Range of variation	1 month to 16 yrs.	2 to 16 yrs.
Average no. of years treated with protamine-zinc insulin	2.1	1.6
Range of variation	1 month to 5 yrs.	3 mos. to 5 yrs.

In order to discover whether protamine-zinc insulin has a harmful effect on the parenchyma of the liver and favors the appearance of hepatitis in diabetics, all the insulin-treated cases in the material were divided up as follows: (1) cases treated with Vitrum insulin only; (2) cases treated with protamine-zinc insulin, possibly with Vitrum insulin also. In the first group, which comprises 18

cases, we find two cases of hepatitis, or 11.1 per cent, in the second group, comprising 151 cases, we find 20 cases of hepatitis, or 13.3 per cent. The two groups show statistical identity.

A study was made of the possible effect on the appearance of hepatitis in diabetics of two further factors, namely, the age of the patient when diabetes was diagnosed and the duration of the diabetes. No difference could be found between patients with diabetes only and patients with diabetes and hepatitis in these respects either.

	Diabetes	Diabetes + hepatitis
Average age of patients when diabetes discovered	37.6 yrs.	38.6 yrs.
Range of variation	1 to 74 yrs.	2 to 67 yrs.
Average duration of diabetes	7.4 yrs.	5.8 yrs.
Range of variation	1 month to 26 y s.	5 mos. to 16 yrs.

As already mentioned, Steinitz observed the diabetes-hepatitis combination more frequently in the winter than during the rest of the year, while Stockinger and Wenzel considered that no such seasonal variation existed. In my material the cases of hepatitis in the groups hepatitis and diabetes and hepatitis only were divided among the various seasons as follows:

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Diabetes & hepatitis ..	1	1	1	—	1	2	2	2	6	3	5	1
Hepatitis	2	2	4	—	1	3	3	1	6	4	4	2

There was a suggested accumulation of cases during the summer and autumn. Whether this indicates a seasonal variation cannot be decided in view of the limited size of the material. Epidemic hepatitis showed a typical seasonal variation, i. e. a definite minimum in June and July and a definite maximum in October and November. That no seasonal variation could be found may be due,

as mentioned above, to the smallness of the material, but, also with regard to the hepatitis-diabetes combination, as claimed by Selander, may be explained by the fact that hepatitis in diabetics is often caused by direct inoculation of virus in connection with the taking of blood samples and that this form of hepatitis by inoculation has a longer and often more varying incubation than epidemic hepatitis. Selander reported the incubation for inoculation hepatitis to be two to three months as a rule, and even as much as six to eight months in certain cases. Assuming an incubation of this length, all my cases of diabetes and hepatitis could have been infected with the hepatitis virus in connection with blood tests. For the time which elapsed between the diagnosis of hepatitis in diabetics and the last blood sample taken before the diagnosis varied between one and seventeen weeks. However, inoculation with the virus of hepatitis in connection with the taking of a blood sample cannot be the only decisive factor in the origin of hepatitis. Only three of the 32 cases in the control group of patients with hepatitis had visited the medical out-patient department of this hospital during the year immediately preceding the onset of hepatitis; as far as could be ascertained none of the remaining 29 patients had undergone blood tests during the year before the appearance of hepatitis. That inoculation with the virus of hepatitis in connection with the taking of blood samples does not suffice to explain the increased incidence of hepatitis in our diabetic patients during recent years, particularly in 1942, appears probable, since not one case of hepatitis has been observed in another category of cases treated in 1942, namely our cases of gastric and duodenal ulcer. This despite the fact that the average number of blood tests was undoubtedly practically as large in the individual ulcer patients as in the individual diabetic patients.

My material supplies no satisfactory explanation of the common combination of diabetes and hepatitis. Possibly diabetics have a certain disposition toward hepatitis. This assumption is supported by the fact that at least some workers (Dalous, Fabre and Valdiguié, Krarup and Iversen) found degenerative changes in the livers of diabetics. Further evidence that this is the case was provided by Rabinowitch, who observed hyperbilirubinemia and hyperurobilinogenuria in diabetics. Hyperbilirubinemia was also observed by Rathery, Polydoridès and de Traverse. In 81 cases of diabetes

mellitus without visible jaundice in my material, I determined the Meulengracht icteric index of the blood and made urobilin and Hammarsten tests of the urine. The results of these investigations are shown in the following table:

Meulengracht icteric index	3—4	5—6	7—8	9—10	11—12
No. of cases	43	27	8	1	2
Urobilin reaction in urine		Neg.	Trace	Pos.	
No. of cases		57	22	2	

The results of the Hammarsten test were negative in all 81 cases. In 13.6 per cent of the cases the Meulengracht icteric index was slightly above normal. On the whole the urobilin content of the urine was normal.

Summary.

1. Twenty-five cases of acute hepatitis occurred in a series of 193 diabetic patients, 18 of them in 1942. The hepatitis rate for 1942 was therefore 9.33 per cent.

2. Hepatitis in diabetics was compared with hepatitis in non-diabetics from several viewpoints. The average age of the patient at the onset of hepatitis was found to be higher in diabetics than in non-diabetics. The difference was only numerical, however. Jaundice remained for a longer time in diabetics than in non-diabetics. This difference too was numerical only. The maximal icteric index was approximately the same in both groups. Likewise, the results of sublimate tests of the feces were on the whole the same for both groups. Hepatic enlargement in patients with diabetes and hepatitis was more pronounced than in patients with hepatitis alone. This perhaps can be explained by the circumstance that diabetes in itself leads to enlargement of the liver. No definite difference could be found between the leukocyte pictures in the two groups.

3. No factor causing a disposition toward hepatitis could be found in diabetic patients. The investigation on this point was based on the level of the blood sugar, the presence of acidosis, glycosuria and albuminuria, the period of time during which the patients had received insulin, the kind of insulin administered — Vitrum insulin or protamine-zinc insulin — the age of the

patient when diabetes was diagnosed and the duration of diabetes. Special emphasis was laid on the fact that protamine-zinc insulin did not appear to have affected the incidence of hepatitis in diabetics.

4. A satisfactory explanation of the common combination of diabetes and hepatitis is not provided by the writer's material. A certain tendency toward hepatitis on the part of diabetics should not, however, be regarded as improbable.

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Studies on the Erythrocytes' Content of reduced Glutathione and its Relation to the Hematopoiesis.¹

By

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(Submitted for publication January 20, 1944).

Stimulated by Hammett's and Reimann's demonstration of the accelerating effect of sulphhydryl compounds on the rate of cell division, Parker & Kracke in 1936 made a study of the blood glutathione distribution in benzene-leukopenic rabbits and found that the reduced glutathione (GSH) measured in blood from the heart, was markedly decreased, and that also the so-called Gabbe's quotient (glutathione in mg per cent/erythrocytes in 1,000,000 per mm³)² was low. Also the bone marrow glutathione was decreased. As the glutathione reduction might have preceded the leukopenia, they advance the hypothesis that the leukopenic effect of benzene may take place by depletion of the normal acceleration factor for cell division in the bone marrow, which appears to be reduced glutathione. In a case of myeloid leukemia they found increased glutathione values in the blood; in a case of lymphatic leukemia no such increase. They suggest that the glutathione plays an important rôle in regulating the production of granulocytes in the bone marrow.

I shall not here discuss in detail the experiments of Hammett and Reimann or the criticism of which their conclusions have been

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² Which I suggest be called: the glutathione index.

the object; except to say that in the course of time a great many observations have been made, which seem to show that sulphydryl compounds play a very considerable rôle in the processes of metabolism associated with the cell division both in plants and in lower and higher animals; even if they probably do not, as originally believed, function as a kind of »mitose hormones».

Some authors have confirmed, — though, to be sure, on the basis of experiments made with a somewhat unreliable technic, — that the glutathione is often markedly elevated in the blood of patients with leukemia, but that at the same time there does not seem to be any certain correlation between the leukocyte figures and the glutathione values. As the sulphydryl group (except the sulphydryl of the proteins) occurs in the glutathione, and considering the biological importance of the latter, these findings are highly interesting and justify further studies of the glutathione changes, precisely in the leukoses. My own preliminary experiments confirmed that the glutathione values, both in myeloid and lymphatic leukemias, often were very high, and also the glutathione index (GSH/Erythr.) markedly increased. I very soon found, however, that before these investigations could be continued it was necessary to examine what influence the often accompanying anemia might have on the blood glutathione level; and a test of Parker & Kracke's results became a natural part of these preliminary investigations.

The method oftenest used for determining the glutathione content of blood and tissues is by iodometric titration in protein-free filtrates. As the glutathione is not present in plasma, the erythrocytes must be hemolysed before the precipitation of the proteins. For the blood filtrates this method, if the procedure is carried out properly, gives correct values as compared with the highly specific glyoxalase method of Schroeder & Woodward, which is based on the fact that glutathione is a specific activator for glyoxalase. That this is so I have been able to confirm myself. With tissue extracts the iodometric titration cannot be used, owing to the presence of other reducing substances; which unfortunately renders a great many earlier studies on glutathione in the tissues invalid.

Though the glyoxalase method is highly specific, it does not get over the difficulty that lies in the precipitation of the proteins. The different precipitation agents give filtrates with somewhat diffe-

rent contents of glutathione. In my own experiments I found that the trichloroacetic acid filtrates always contained a little less GSH than the metaphosphoric and sulfosalicylic acid filtrates. The two lastnamed gave very nearly identical results. I have, like Fujita & Numata preferred to use metaphosphoric acid, for the reason, among others, that sulfosalicylic acid sometimes gives opalescent filtrates, which makes the titration difficult. In the experiments made with the object of testing Parker & Kracke's results I used both sulfosalicylic and metaphosphoric acid filtrates, however; because these authors used sulfosalicylic acid.

In other respects the method usually employed for the determination of glutathione in blood filtrates has been a slight modification of Fujita & Numata's. Attempts to estimate oxydised glutathione (GSSG) before and after reduction of the filtrate with hydrogen in statu nascendi by the addition of zinc dust to the acidous filtrates had to be abandoned, because the method gave very irregular results and oftenest no increase at all of the GSH value. And in fact, according to more recent investigations, by Dohan & Woodward, with an electrolytic method of reduction, no GSSG occurs in the blood.

As standard method I have therefore used the following:

Immediately after the blood-taking 1 cm³ of blood (venous blood, oxalated blood) is blown into 5 cm³ of distilled water in an Erlenmeyer flask, and after the flask has been shaken gently is allowed to stand for two minutes in order that hemolysis may take place. Then 4 cm³ of 5 per cent metaphosphoric acid is added and the mixture is filtered. Of the filtrate, 2 cm³ are used for the titration, which is done in small test tubes at 0° C. as follows. A 0.2 cm³ portion of freshly prepared 5 per cent potassium iodide solution and 1 drop of 1 per cent starch solution (in saturated NaCl) are added, and the mixture titrated with 1:100 N KIO₃ until the first faint blue color persists. Always duplicate titration. Counting of the erythrocytes and such other hematologic examinations as may be needed are always carried out on the sample of blood.¹

I have controlled this method with the glyoxalase method, carried out as indicated by Schroeder & Woodward with excep-

¹ About the reliability and exactitude of the iodometric method for titration of reduced glutathione in acidous filtrates, with starch as indicator see the numerous works on these questions (6, 19, 20, 22, 36).

tion that I used metaphosphoric acid instead of sulfosalicylic acid extracts; which, however, according to those workers does not make any difference so far as the technic is concerned (Table 1). I also found it unnecessary to add more than half the quantity of bisulfite stated by them in order to bind the methylglyoxal. This saves considerable quantities of iodine. Furthermore it is my impression that it is necessary to wash out the acetone yeast somewhat more thoroughly with water than indicated by Schroeder & Woodward. The methylglyoxal was prepared in crystalline form by oxidation of acetone with selenium oxide, because neither glyceraldehyde nor dihydroxyacetone could be obtained. In plotting the standard curve I have made use of a somewhat greater number of glutathione concentrations than Schroeder & Woodward, but otherwise I have followed their technic closely, and the method now works satisfactorily.

Table 1.

Simultaneous Determination of Glutathione Content in different Blood Samples by Iodometric and Glyoxalase Methods.

Glyoxalase Method	Iodometric Titration
26 mgr/per cent	25.4 mgr/per cent
44	41.5
32	31.5
31	30.2

It is important that the blood should be precipitated as soon as possible after taking, as otherwise the GSH may become transformed into oxidised glutathione (GSSG). But after the protein has been precipitated it may be left standing for twenty-four hours without any measurable decrease taking place in the glutathione values; which has been of importance for the examination of samples from patients from different hospital services.¹

In some cases I examined the plasma for glutathione by the glyoxalase method, but never found any measurable amount.

¹ For permission to use suitable cases from other hospital services I thank especially Prof. E. Meulengracht, M.D. and Prof. H. C. Gram, M.D.

I. Benzene-leukopenic Rabbits.

With exception of the experiments of Parker & Kracke referred to above, I have found no reports of studies of the glutathione content in blood under the influence of benzene; which is strange considering the important conclusions come to by these two authors as the result of their researches. Like them, I used rabbits in my

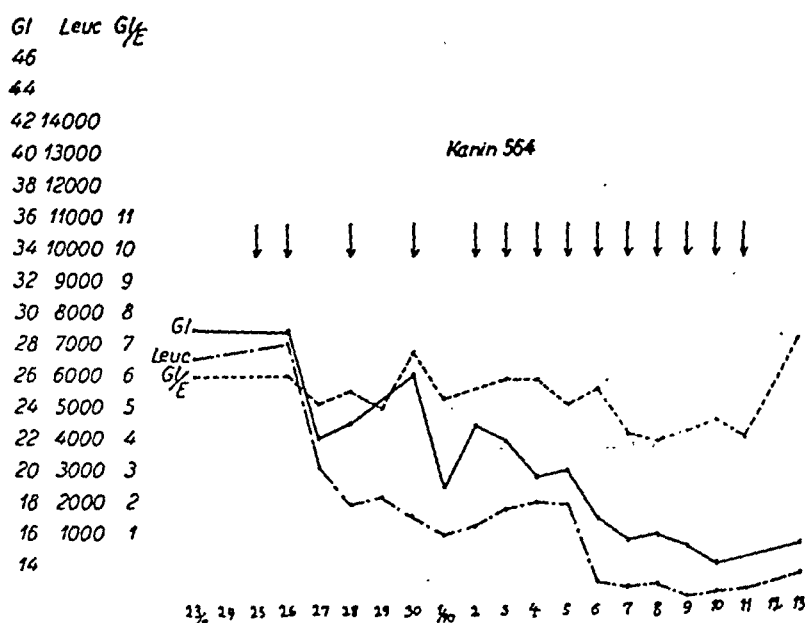


Fig. 1. — The arrows point to the dates of the subcutaneous benzene injections (3.2 cm³ of benzene in 3.2 cm³ of olive oil).

experiments. I first tried to determine the normal values for GSH and glutathione index in these animals, but found that the daily blood-taking resulted in the erythrocyte values during the first days showing a slightly falling tendency, whereupon they became stationary at a slightly lower level. From then on, the values found in the individual animals remained fairly constant from day to day, while there might be rather great variations from one animal to another. Of course, the use of such rabbits introduces a source of error, by the fact that one works with animals that evidently are under the influence of chronic bleeding, however slight. But as it in these experiments is a question especially of following

the variations for some length of time, the normal values are as a matter of fact of minor importance; and as the variations occurring are great compared with those resulting from the daily blood-taking there must be justification for leaving such a potential source of error out of account.

The experiments were carried out in the manner that the animals were first controlled for some time, until the glutathione and erythrocyte values were constant. Then a mixture of equal parts of benzene and olive oil was injected subcutaneously, daily or at different intervals of time. Also the dosis was varied, in order to provoke if possible, either simple granulocytopenia or granulocytopenia accompanied by erythrocytopenia. The experiment was in all cases gone on with until the animal died. Glutathione determinations, counting of erythrocytes and leukocytes, hemoglobin measurements, and in some cases hematocrit determinations, were made during the whole experimental period, as a rule every day. As regards the glutathione determinations the results were all alike, in spite of the various technic employed in the different experiments. As long as the red blood picture did not change there were no glutathione changes either; nor, consequently, any change in the glutathione index, even if the leukocyte count had sometimes before then fallen to a very low level. As soon as the erythrocyte values began to fall, also the glutathione values decreased in a more or less parallel manner, so that the glutathione index remained fairly constant during the whole experimental period (Fig. 1). Only just before death was there in a few of the experiments an increase in the glutathione and the index.

II. Roentgen-leukopenic Rabbits.

To complete the above experiments, I made similar studies on rabbits before and after exposure of them to all-over irradiation with roentgen (160 kv., 4 ma., distance 50 cm, intensity 8/r. min.). Seven animals in all were examined, which were exposed to doses of respectively 250, 500, 800, 1000, 1200, 1500 and 2000 r). The lowest doses caused no changes whatever either in the hematologic state or in the glutathione values. The higher doses, as expected, provoked leukopenia and also had some effect on the erythrocyte

values. Otherwise the results were exactly the same as in the benzene experiments. The depression of the leukocyte values had no effect on the glutathione values or the index; the former followed the erythrocyte values, so that the index remained fairly constant. Fig. 2 shows the curves for the rabbit which was exposed to irradiation with 800 r.

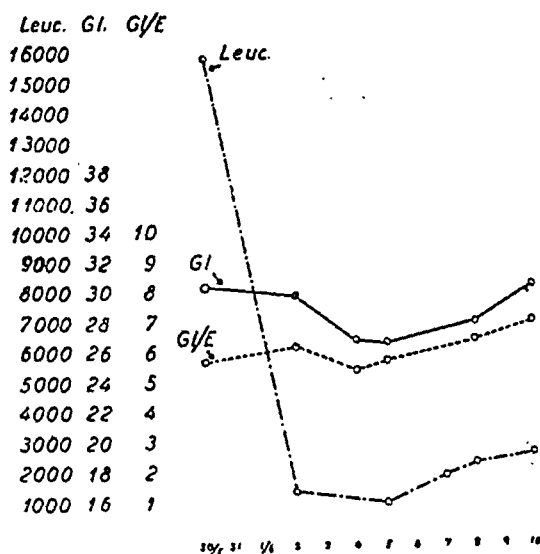


Fig. 2. — Curves showing leukocyte values, glutathione values and glutathione index for rabbit exposed on June 1st to all-over irradiation with 800 r.

III. Hemorrhagic Anemias.

With regard to the blood glutathione in hemorrhagic anemias a good deal is already known. Shortly after a hemorrhage of any magnitude there is an acute glutathione decrease corresponding to the fall in the erythrocyte values; but afterwards the glutathione values rise more rapidly than the erythrocyte count, so that the glutathione index for some time is increased. Later, when the regeneration is getting more or less complete, the index again approaches the normal (2, 7, 21, 35). These observations I have been able to confirm by studies on 3 rabbits examined before and for some time after a bleeding (see Fig. 3). I have not been able to demonstrate any relation between glutathione value and reticulocyte count.

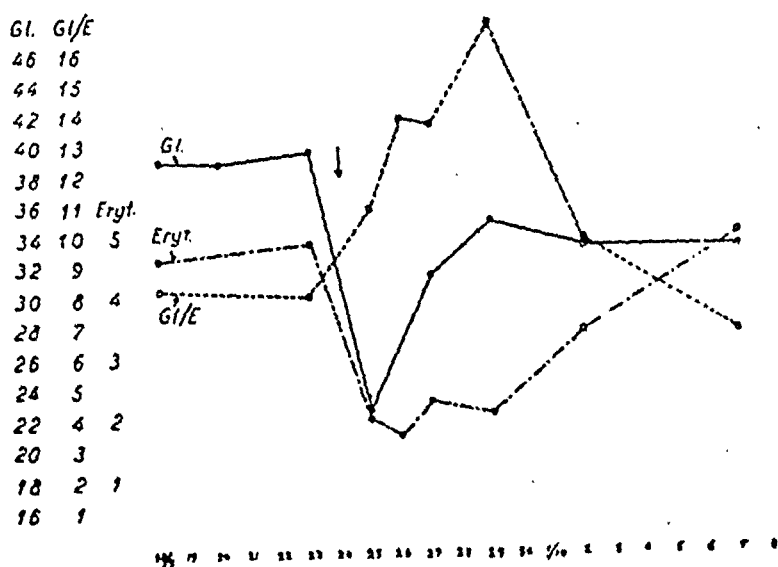


Fig. 3. — Rabbit. The arrow points to the date of bleeding.

IV. Experimental Leukocytosis.

In a number of rabbits I attempted to provoke leukocytosis by intravenous injections of sodium nucleinate, suspension of colibacilli, egg-albumin, sodium taurocholate and human serum, but did not succeed in provoking excessive leukocytosis, only moderate increases up to about 30,000. In none of these cases could any change in the glutathione index be demonstrated with certainty.

The results of these preliminary experiments do not confirm Parker & Kracke's suggestion of the glutathione as a factor of essential importance for the activity of the granulopoiesis. In none of the experiments did there occur any variations of the glutathione values or the glutathione index that could be supposed to have any relationship to the intensity of the production of granulocytes. Much rather might there from these results be supposed to be a certain relation between the intensity of the erythropoiesis and the amount of glutathione in the blood. In the experiments in which it must be presumed that the marrow had been injured and the cell production in consequence reduced, — as in the benzene — and roentgen experiments, — the fall in the glutathione values was more or less parallel to the fall in the number of erythrocytes, whereas there was no variation in relation to the number of granulocytes. At the same time the glutathione index remained constant;

which is to say that the individual red cell contains its normal amount of glutathione. In contrast to these forms of anemia, the hemorrhagic anemias show a greatly increased glutathione index during the period of regeneration. It would thus seem as if there were a certain relationship between the glutathione content of the erythrocytes and the intensity of the regeneration of the erythropoieses. The finding, by Gabbe and by Bach & Bach, of a similar much increased glutathione index in rabbits treated with phenylhydrazine, as well as certain studies on human subjects, seem to support this hypothesis.

V. Glutathione in Man.

The statements of the different authors respecting the normal glutathione values in man vary a little, depending on the method employed in their studies. But as I have found the iodometric procedure which I used, and the specific glyoxalase method, to give almost identical results, the values stated below will at least be correct for the quantities present in the filtrates after precipitation with metaphosphoric acid.

It has been shown that sex, ingestion of food and physical exercise have no influence on the glutathione level (2). On the other hand, Rosenberg has found a slight increase of the values in old age. In such investigations it is of course of the greatest importance to consider the erythrocyte count and determine the glutathione index, or to do hematocrit determinations and calculate the content per 100 cm³ of red cells.

In 66 subjects of both sexes, in ages from 17 to 60 years, I found on the average 33.9 mg/per cent of glutathione, calculated in full blood, with a standard deviation of 4.39 and a standard error of ± 0.173 . The glutathione index was in the average 7.5; the deviation here 1.03, the standard error ± 0.13 . The subjects were persons who were feeling well, whose sedimentation rate was normal and who were not affected with anemia (the females: over 400,000,000 erythrocytes; the males: over 450,000,000). There was no positive difference between the two sexes and no relation to stature or body-weight; but of course the material is too small to warrant any absolutely sure conclusions in these respects. At the same time I examined 16 normal subjects in ages from 60 to 98 years.

Glutathione (GSH) was on the average 31.6 mg/per cent; the glutathione index 7.1. Neither in these cases is there any demonstrable difference. In some subjects the values examined at intervals of some days were then found very constant.

Pernicious Anemia.

A few earlier determinations exist, of the blood glutathione in patients with pernicious anemia. In these, there was invariably

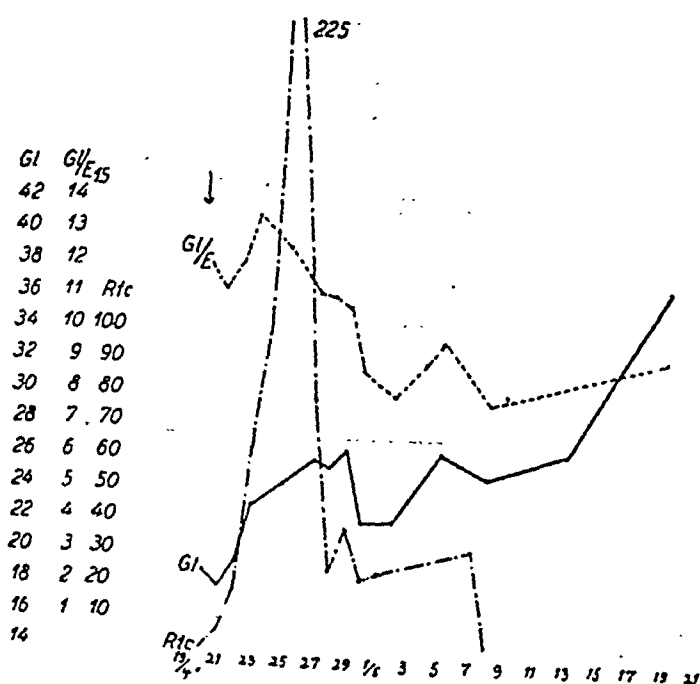


Fig. 4. — Pernicious anemia. The arrow indicates the time at which the specific treatment was instituted.

in the untreated cases found remarkably high GSH values in proportion to the number of erythrocytes; in other words a very high glutathione index. Under the specific therapy the index gets lower as the erythrocyte count gradually increases (2, 34).

My material includes 4 examined patients with pernicious anemia. Their glutathione index before treatment was instituted was on the average 13.7, and highest in the cases in which the number of erythrocytes was lowest. As soon as the specific therapy was instituted the glutathione began to increase at the same time as the erythrocyte values, but more slowly than these, so that the glutathione index showed an even decrease. In Fig. 4 are presented

the curves from a typical case. There does not seem to be any relationship at all between the reticulocyte- and the glutathione values. The variations in the GSH/vol. per cent show a curve similar to that of the glutathione index. In one patient the latter index rose still further during the first period of the treatment, but otherwise the picture was the same in all the four patients.

Hemorrhagic Anemia in Man.

Of such cases I have examined only two: one after a hemorrhage from an erosion of the cervix uteri (red cells, 3,510,000; glutathione index 10.4); the other after hemorrhage from a tonsillectomy (red cells, 2,200,000; glutathione index 10.7). As might be expected, the glutathione index was high in both. The conditions as regards the glutathione in cases of hemorrhagic anemia are the same in man as in rabbits, and have been found so also by the other authors who have written on the subject; I have therefore deemed it unnecessary to carry my own investigations on this point any farther.

The result of these first investigations is thus that in rabbits there occurs under the influence of benzene intoxication a decrease in the glutathione content of the blood, which has no relation however, to the decrease in the number of granulocytes in the latter, but, on the contrary, seems to be parallel to the decrease in the number of erythrocytes, so that the glutathione index remains fairly constant. Exactly the same was found in the rabbits that had been exposed to large doses of all-over irradiation with roentgen. In rabbits that had suffered a considerable loss of blood, the glutathione at once fell at the same rate as the erythrocytes, and then rose relatively faster than these during the regeneration; so that the glutathione index for a time became distinctly increased and only approached normal values as the erythrocyte count gradually rose to normal figures. Experimentally provoked leukocytosis caused no alteration of the glutathione content in the blood.

In man, the glutathione index in pernicious anemia is very high during the untreated stage of the disease. Under the influence of the specific therapy it falls, and approaches normal values as the

erythrocyte count gradually reaches normal figures. In cases of hemorrhagic anemia in man, the index is moderately high, the same as in rabbits.

Nothing has thus been found in these experiments to indicate a relation between the intensity of the granulopoiesis and the level of the blood glutathione. On the other hand observations were made which point to a certain relation between the amount of glutathione in the erythrocytes (the glutathione index) and the activity in the bone marrow; though it is not possible to say, as yet, whether these variations in the glutathione level are entirely secondary in relation to the changes in the erythropoiesis or whether they are of any primary significance for the intensity of the latter. The problem is interesting on the background of the many reports about the rôle of the sulphydryl compounds in the processes of cell-division. That the investigations related in the preceding pages make it appear as if the glutathione were of no significance for the activity of the granulopoiesis must of course be taken with a certain reservation; because the possible variations that might be thought to occur in the intensity of the latter may perhaps not come to expression in the glutathione content of the erythrocytes, but in that of the granulocytes. About the glutathione content of these, nothing exact has been found yet; but at any rate it is certain that the amount, simply on account of the small number of these cells, must be very small compared with the large amount contained in the erythrocytes. Therefore, small variations in the number of granulocytes in the blood will not make themselves felt when the glutathione content of the blood is determined. That great variations, as we see in the leukemias, may nevertheless cause variations in the glutathione content, will be made the object of studies that will be presented in a later paper. But at all events the results of the investigations related in the present work show that the variations observed in the blood glutathione under the influence of benzene cannot, as believed by Parker & Kracke, be taken as supporting evidence for a theory of the glutathione playing a rôle in regulating the granulopoiesis.

Summary.

As sulphydryl compounds are supposed to have great influence on the course of the chemical processes in connexion with cell-division, Parker & Kracke interpret the results of their studies on the blood glutathione values in benzene-leukopenic rabbits as indicating that the blood glutathione is a normal accelerative factor for cell division in the bone marrow, because injections of benzene into the animals resulted both in leukopenia and in depletion of the reduced glutathione in the blood; and they would ascribe to the blood glutathione an important rôle in regulating the granulopoiesis.

The author has tested these experiments and shows that while there no doubt in benzene leukopenic rabbits is a decrease of reduced glutathione in the blood there is no parallel between this and the number of granulocytes, but rather a parallelism of the glutathione decrease and the erythrocyte values; these two values falling at about the same rate, so that the individual red cell's content of reduced glutathione (glutathione index) remains fairly constant. Similar results were obtained in rabbits that had been made leukopenic by all-over irradiation with roentgen. In experimentally produced leukocytoses there is no change in the blood's content of reduced glutathione. In hemorrhagic anemias in rabbits there is at first an immediate fall in the glutathione values, but afterwards a rapid rise, so that the glutathione index for a time is considerably increased. In hemorrhagic anemias in man something similar is the case. Also in pernicious anemia in man there is in the untreated stage increased glutathione index, which under the specific treatment returns to about normal values.

On the basis of these researches and other results from the literature, the author points out that while a relation between the glutathione content of the granulocytes and the activity of the granulopoiesis is not altogether excluded, a number of circumstances point to a connexion between the activity of the erythropoiesis and the erythrocytes' content of reduced glutathione. His studies on this subject will be continued, especially with a view to the leukoses, in which the blood's content of reduced glutathione can be very large.

These researches were begun in the Blegdam Hospital in Copenhagen and in the Niels Steensen Hospital in Gentofte. I am indebted to the chiefs of these two institutions for the excellent facilities afforded me there for my work.

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The Circulation in Fallof's Tetralogy.

By

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(Submitted for publication February 5, 1944).

The understanding of the pathogenesis of congenital pulmonal stenosis with a defect in the septum interventriculorum is closely connected with the understanding of the abnormal circulation in this affection both as regards the quantitative and the qualitative conditions. Therefore, with a view to determining the volume of that part of the venous blood which perfuses the lungs and the remaining part which passes through the septum defect into the aorta, I have performed a series of examinations on a patient with typical morbus caeruleus (the tetralogy of Fallof). Such examinations have hitherto been performed to but little extent (Raab 1924; Dautrebande, Marshall, Meakins 1929).

For the sake of completeness the abnormal circulation shall be recapitulated briefly. The blood from the right atrium during the diastole passes to the right ventricle whence, during the systole, it is pumped partly through the stenosed pulmonal artery, partly through a defect in the septum interventriculorum, into the aorta. The site of the aortal ostium in relation to the septum may vary, often the aorta is found riding on the septum corresponding to the defect so that it will be possible from the aorta to look down into the right as well as into the left ventricle. Thus the aortic blood consists partly of venous blood (via the septum) and partly of oxygenized blood which from the lungs has passed through the left atrium and the left ventricle.

Thus the right ventricle will be charged with some of the work of the left ventricle, having to discharge its content against the relatively strong resistance in the greater circulation. The consequence thereof is an often considerable hypertrophy of the right ventricle which, in casu, presented itself electrocardiographically as a marked axial deviation to the right and, roentgenologically, by a somewhat enlarged heart with hypertrophy particularly of the right ventricle (which measured 30 by 16 by 7.5 cm).

To the difficult transport of oxygen due to the reduced pulmonary circulation the organism responds by compensatory polyglobulism. In this patient the Hgb. was found to be 175 per cent (haemometer sicca) and erythrocytes about 9.3 mill.

Technic and Therapeutic Results.

The patient having been practised in the technic the arterio-venous utilization was determined 5 times with Grollman's acetylene method and made up after Lindhard's formula. The examinations were performed on the fasting patient in dorsal position. He was carried to the examination room, because, after the fairly long walk from the sick-room, it proved to be difficult to reduce the metabolism to the vicinity of the standard value. For the calculation of the minute volume the metabolism was determined with the Douglas bag method in a 15 minute period. (In 1 case Krogh's metabolism apparatus was employed). Analyses of samples of air were carried out with Haldane's apparatus modified by Krogh. The results are recorded in the subjoined table.

Patient K. E. G. Case report No. 963/43, æt. 26 years.

No	Date	O-consumption min. (cm ³)	Utilization (cm ³ per liter)	O-consumption utilization	=pulmonal flow
1	25/8	219 (Krogh)	92	2.39	
2	4/9	216 (Douglas)	80	2.70	
3	28/9	231 (")	93	2.48	
4	12/10	244 (")	97	2.52	
5	14/10	242 (")	105	2.30	

In the experiments 3 and 5 determination of metabolism, arterial puncture and utilization determination were performed in immediate succession. The arterial blood was drawn from the

femoral artery with the cannula mounted on a glass syringe whose piston was greased with paraffin oil. The dead space was filled with a concentrated, boiled out oxalate solution. The blood (about 15 cm³ in all the experiments) was at once transferred to a small quicksilver recipient, and the mouth and boring of the cock were closed with quicksilver. The sample was placed in the refrigerator until the analysis could be made some hours later.

The determination of the oxygen content of the blood was performed with a Van Slyke apparatus in the usual manner (duplicate determinations).

The following values were found:

Oxygen capacity: 32.1 vol. %.

Oxygen content of arterial blood in exp. 3: 24.8 vol. %. Degree of saturation: 77 %.

» » » » » » 5: 24.7 vol. % Degree of saturation: 77 %.

The oxygen content of the mixed venous blood must thus be:

In experiment 3: $0.95 \times 32.1 - 9.3 = 21.2$ vol. %,

» » 5: $0.95 \times 32.1 - 10.5 = 20$ vol. %.

as it is presumed that the blood in the lungs attains a degree of saturation of 95 per cent.

The oxygen content of the volume of blood flowing through the aorta in 1 minute may then be expressed by the following formula:

$$L \times \text{total capacity} \times 0.95 + X \times a = (L + X) \times b,$$

L being the pulmonic flow, X the volume sought for which passes through the septum defect per minute, a being the oxygen content in the mixed venous blood, b the oxygen content in the arterial blood. The values found being inserted, the following figures are obtained:

	X (L/min.)	L (L/min.)	X + L = minute volume of greater circulation	$\frac{X \times 100}{(L + X)}$
Experiment 3	3.93	2.48	6.41	61 %
» 5	2.84	2.30	5.14	55 %

Discussion.

The presupposition for the correctness of the reported results is (1) that the blood is saturated (about 95 per cent) in the lungs, and (2) that there are no abnormalities causing reflux of blood from aorta to pulmonary artery.

To (1). Neither clinical nor roentgenological signs of stasis being demonstrated, the diffusion may be regarded as reliably undisturbed. Nor was any emphysema detected, where the increased residual air and the bad mixture of the inspiration air might occasion a partial decrease of the oxygen tension of the alveolar air and, consequently, insufficient saturation of the pulmonary blood.

If the pulmonic flow is reduced, which may be anticipated after the whole nature of the affection, it must moreover be regarded as exceedingly probable that complete saturation is attained.

To (2). Here we chiefly think of ductus arteriosus persistens which would render the application of the acetylene method impossible, since the chief condition here is that acetylene-containing blood does not flow back to the lungs within the time of experiment. If that does happen, the determination of utilization will yield too high figures, and the minute volume will be calculated too low. Even though the combination of the two abnormalities must be regarded as rare and signs of a ductus arteriosus persistens were not detected roentgenologically, the possibility of its existence must be said to impart some uncertainty to the results. A patent foramen ovale will scarcely give rise to mistakes, since the blood stream through it must be assumed to go from right to left, the right atrium during the diastole receiving about twice as much blood as the left.

The pulmonary flows found, which indeed amount to about half of what is found in the normal, quite agree with Lindhard's results. This author examined a 23 year old patient with morbus caeruleus, and in 12 experiments found 1.37 to 2.51 L (averagely 1.89 L). These somewhat lower figures might indicate that the stenosis was more pronounced in his patient than in this case. The clinical picture also seems to show this, for Lindhard's patient was scarcely able to walk, whereas mine could perform moderate muscular work (400 kgm/min. in 10 minutes).

As is seen, the figures derived from experiment 3 are higher than in experiment 5. In experiment 3 the patient was very restless, struggling under palpation of the genito-femoral sulcus, and prior to the experiment a vain attempt at puncturing the brachial artery had been made. Experiment 5 went off smoothly. On the other hand the percentual part of the total minute volume passing through the septum defect was fairly the same in the two experiments, thus showing that half of the minute volume of the greater circulation passes unoxygenized through the defect.

The cyanosis. The cyanosis of hands and feet was pronounced when the patient was resting, whereas his face was rather erythrocyanotic. On performing moderate muscular work (300—100 kgm/min. on Krogh's ergometer bicycle) it increased very considerably everywhere, his ear lobules, lips and hands becoming blackish-blue, his face greyish-blue. The exercise gave rise to palpitation, objective and subjective dyspnea, precordial sensations but no precordial pain, which was reported always to occur when the patient performed some harder work.

The cyanosis depends of the colour of the blood, and it appears when the mean oxygen deficit of the capillary network has reached a certain magnitude. The mean oxygen deficit is not known, and C. Lundsgaard (1919) therefore uses the sum of the deficit on the arterial and on the venous side of the capillary network, finding that the cyanosis appears when this sum exceeds 13 vol. %. It may here be inserted that it will be more correct to express the deficit sum as a relation of the total capacity, since the colour of the blood may not be due to the reduced hemoglobin alone, though also to the amount of oxyhemoglobin admixed to it. In Lundsgaard's experiment the total capacity was 20.8 vol. %, and the deficit sum of 13 vol. % thus corresponds to 63 per cent of the total capacity. In my experiments the oxygen content of the blood of the venae cutaneae is not known, but if the deficit of mixed venous blood is used instead, the mentioned sum during rest is 59 per cent of the total capacity ($7.4 \times 100: 32.1 + 11.5 \times 100: 32.1$), that is to say, just close to the limit of cyanosis.

During work this becomes still more distinct. During an exercise as that performed by the patient the utilization in a normal person would rise from about 5 to about 8.5 vol. %. In the patient it will correspond to a rise from about 10 to about 17 vol. %, if the

minute volume increases in a proportion corresponding to the magnitude of the work. On applying the mentioned equation $L \times 31 + X \times a = (L + X) \times b$ to the calculation of the volume of blood which has passed through the septum defect, an idea of the arterial oxygen saturation (b) may be obtained, if $a = 14$, $L = 42$, $X = 58$, since the proportion between L and X according to the figures found in resting must be about 42: 58. b then becomes about 21 vol. %, and the arterial deficit, about 10 vol. %. The deficit sum during work will thus be 84 per cent of the total capacity or considerably above Lundsgaard's limit of cyanosis.

Even though absolute importance with regard to the cyanosis of this patient cannot be attached to these values, they nevertheless afford an idea of the primary responsibility of the insufficient arterial oxygen saturation for the cyanosis partly during rest and partly during work.

The figure, 17 vol. %, applied to the venous deficit during work certainly is too low too, for under circumstances as these which present pronounced decompensation (dyspnea, precordial sensations, extreme cyanosis) it must be anticipated that the minute volume cannot be increased in proportion to the work. Suggestive in this respect is the venous pressure which, on being measured in 1937 during the patient's stay in the internal department B of the Rigshospital (case report 488/37), amounted to 170 mm hydrostatic pressure or about double the normal. Thus the flow from the right atrium to the left ventricle must have been made difficult, probably on account of insufficient discharge during the systole caused by the many difficulties of the right ventricle. It must work against the resistance of the greater circulation, the conditions of discharge to the aorta may possibly be difficult and, finally, the oxygen supply of the hypertrophic myocardium — especially during work — certainly is compromised in a marked degree. Thus the utilization will increase, the blood which through the septum defect is mixed with blood from the lungs will be still poorer in oxygen and the arterial oxygen content will be further decreased.

About the peripheral component of the cyanosis, i. e. the decreased rate of flow in the cutaneous capillaries and the increased deoxidation due to it nothing can be said with certainty. In my opinion, however, it is natural to think that the minute volume during rest and particularly during work greatly consists of the

blood from the muscles and other organs with great requirements of oxygen, whereas the irrigation of the skin is decreased as much as possible.

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Some Investigations on the Influence of Diet on the Ascorbic Acid Content in Serum.

I.

By

INGRID EBBESEN and MOGENS RASMUSSEN.

(Submitted for publication February 4, 1944).

In the present paper a large material is produced of ascorbic acid determinations in serum from normal persons and from patients suffering from sciatica or other diseases, which cannot be supposed to influence the concentration of serum ascorbic acid. The investigations, which have extended over 16 months show that the ascorbic acid content in serum is subject to typical seasonal variation. The ascorbic acid content in persons examined within the same month varies considerably. The knowledge of the fluctuations in normal persons at different times of the year is a necessary basis for estimating investigations on serum ascorbic acid in special morbid conditions, e. g. peptic ulcer, which will be the subject of an examination in a following paper. It is possible to demonstrate a close relationship between the ascorbic acid content in diet and in serum.

The direct reason of these investigations is a work by Ottsen and the opposition by Dr. phil. P. Brandt Rehberg at the public defence of this thesis (1942). Ottsen could not prove any relation between the ascorbic acid concentration in serum and the ascorbic acid content in diet. His material was 406 normal persons (including 70 nurses and 87 feeble-minded children). For the first two groups analyses of diet have been carried out for

three or four weeks; in the last group the amount of ascorbic acid supplied has been calculated from very accurate anamneses of diet. For each person two determinations of ascorbic acid have been made. A relation between the amount of vitamin C supplied through the diet and the ascorbic acid content in serum could not be proved with certainty in any of the three groups. By means of Lund and Lieck's method Holger Nielsen (1938) and Trier (1940) have found evident seasonal variation in the content of serum ascorbic acid. By rough anamneses of diet no disagreement between the ascorbic acid concentration of serum and the estimated C-content of diet was found in any case. Emmerie and v. Eekelen (1937), too, have found distinct seasonal variation. Several investigators have demonstrated increase in serum ascorbic acid after intake of vitamin C, for instance Farmer and Abt (1936), Herlitz (1928) and Bræstrup (1939). In the thesis of the latter the following experiments are mentioned: 5 patients are charged with 40 to 50 mg ascorbic acid per kg body weight. 4 to 6 hours after the administration an increase in the content of serum ascorbic acid of 0.40 mg per cent was found. The patients had not reached the initial values until 48 hours after the administration. Finally Crandon, Lund and Dill (1941) ought to be mentioned. They have carried out a self-experiment with C-free diet during 6 months. After 42 days of C-free diet the ascorbic acid content in serum fell from 1 mg per cent to 0 mg per cent. The first symptoms of scurvy did not show until after 4 months in the shape of lassitude, loss in weight and circumpillar keratosis. After 6 months delayed wound healing appeared.

The accuracy of the method employed and the influence of different factors on the result. Farmer and Abt's method, as described in Bræstrup's thesis, has been employed. A few modifications were introduced, however, serum being used instead of plasma. Besides double amounts of serum, metaphosphoric acid and water were taken, which made it possible to make three titrations on each sample. The titrations were carried out with day-light bulb in a dark-room.. The blank values were all about 0.005 to 0.007. Experiments on the accuracy of the method, in which known solutions of ascorbic acid in 2.5 per cent metaphosphoric acid were titrated, have been carried out. The deviations did not exceed 0.05 mg per cent. In the triple titrations the same result was generally obtained. The deviations practically never exceeded 0.08 mg per cent, which corresponds to one division on the burette. The results are computed as the average of all three titrations, from which is deducted the average of at least 6 blank values.

The blood samples were collected partly after the breakfast of the patients, partly 1 $\frac{1}{2}$ to 4 hours after the mid-day meal. Blood

samples were not collected from patients which on the day of the experiment had eaten fruit before the veni-puncture. As the patients' dinner generally contains potatoes, the ascorbic acid values might possibly be higher than the fasting values. According to Dagulf (1939) and the statement made by other authors the daily supply of ascorbic acid amounts to about 60 mg in the C-richest months and in the C-poorest months to about 15 mg. From this it is estimated, that the dinner of the patients hardly contains more than 50 mg ascorbic acid. Several authors have demonstrated an increase in serum ascorbic acid after charging with 300 to 700 mg ascorbic acid. Lund (1939) for instance finds an increase in the content of serum ascorbic acid of 0.5 mg per cent, 1 ½ and 4 hours after the oral administration of 700 mg ascorbic acid. After this it does not seem likely that an administration of 50 mg ascorbic acid would give an increase which could be proved with certainty.

In order to examine this question 10 experiments were undertaken in which the serum ascorbic acid was determined fasting and 1 ½ and 4 hours after oral administration of 50 mg ascorbic acid to the completely healthy persons. It was not possible to demonstrate any increase in the content of serum ascorbic acid. On account of the small material it is not possible to draw any definite conclusions, but the results seem to indicate that it is hardly important in these investigations, that part of the blood samples were collected 1 ½ to 4 hours after the patients' dinner.

Some experiments have been carried out to examine the stability of ascorbic acid in blood samples (a), serum samples (b), and in the metaphosphoric acid filtrate (c).

a) In 10 experiments serum ascorbic acid was determined immediately after the blood collection and after the blood samples had been standing in the icebox for 24 hours before centrifuging. In one case the deviation was beyond the limit of error, viz. 0.09 mg per cent. The experimental values are plotted in fig. 1.

b) By the examination of three samples, kept as serum in the ice-box for 24 hours, in two cases a decrease of 0.28 mg per cent was found, after which further examinations were not undertaken.

c) The metaphosphoric acid filtrate was kept in the ice-box for 24 hours. In 5 cases out of 14 a distinct decrease was found. On the other hand determinations in the metaphosphoric acid filtrate left for 4 hours showed deviations within the limit of error.

Table 1.

Determinations of serum ascorbic acid immediately after the blood collection and after standing of the blood in ice-box for 24 hours before centrifuging.

0 hours	24 hours
1.52 mg per cent.	1.55 mg per cent.
0.52 »	0.45 »
0.80 »	0.78 »
0.76 »	0.69 »
0.95 »	0.88 »
0.64 »	0.60 »
0.56 »	0.57 »
0.59 »	0.57 »
0.27 »	0.28 »
0.28 »	0.28 »
1.09 »	1.00 »

In consequence of these results the blood samples were generally analysed immediately after the blood collection and always within 24 hours. The titrations were always carried out as soon as possible after the precipitation, two hours after at the latest.

The first 100 determinations were carried out at the Biochemical Institute, Aarhus, by civil engineer Kirsten Volqvartz, the rest by the authors, partly at the Biochemical Institute, partly at Aarhus County Hospital.

Own investigations.

The investigations were commenced in November 1941 and completed in April 1943. During September 1942 no analyses were taken owing to the illness of the authors. In all 650 ascorbic acid determinations on 346 persons have been undertaken. All the subjects have had ordinary plain diet and persons that have eaten plenty of fruit have been included, whereas patients who have had pure ascorbic acid have been omitted. In 25 cases 0 mg per cent ascorbic acid was found. These values mainly occurred during spring. None of the patients showed symptoms of scurvy. The average values of ascorbic acid for the patients investigated appear from Table 2 in which the numbers of determinations of serum ascorbic acid, the average in mg per cent and the standard devia-

Table 2.

Number of determinations of serum ascorbic acid, their arithmetic average and the standard deviation for the separate months.

	number	average in mg per cent.	standard deviation
19/11—19/12 41	44	0.32	0.24
14/1 —28/2 42	32	0.33	0.24
March »	31	0.33	0.25
April—May »	20	0.15	0.11
June »	27	0.04	0.03
July »	41	0.31	0.31
August »	71	0.77	0.32
October »	55	0.62	0.35
November »	54	0.38	0.21
December »	43	0.33	0.19
January 43	68	0.28	0.14
February »	50	0.23	0.13
March »	68	0.20	0.13
April »	47	0.14	0.10

The standard deviation has been computed from the following formula

$$\sigma = \sqrt{\frac{\sum X^2 p}{n} - M^2}$$

tion for each separate month are given. Table 2 shows that the lowest average 0.04 mg per cent is found in June and the greatest 0.78 mg per cent in August. As appears from Table 2 there is a definite seasonal variation, the values of serum ascorbic acid falling in the course of spring until a minimum is reached in June, followed by a considerable increase in July and the beginning of August. The season curve found corresponds approximately to the variation described by other authors in this country. By the examination of 169 children suffering from otitis media Bech Mathiesen (1942) thus finds a quite similar shape of curve with a minimum in June. Trier and Holger Nielsen both find a minimum in May. The rather late increase in our material is perhaps due to the late arrival of spring in 1942. By considering the values for each month it is seen from Table 2, that the standard deviation is very big, especially in July, August and October.

Examination on the serum ascorbic acid for normal persons after the intake of vitamin C.

The results of the examination of serum ascorbic acid in 6 persons are presented. The individuals in question have eaten the ordinary plain diet of the hospital; the addition of ascorbic acid has been varied by the administration of this substance in different ways either as fruit or as pure ascorbic acid by mouth. (We have used tabl. Ido C, 1 tabl.: 25 mg ascorbic acid). For each patient information of the diet and a graphic representation of the variation of the serum ascorbic acid is given. Abscissa axis: time in days. Ordinate axis (left side): serum ascorbic acid in mg per cent. Ordinate axis (right side): ascorbic acid added to the diet in mg per day. Pure ascorbic acid is reproduced as \square , while fruit is reproduced as \blacksquare and has been valued by estimation.

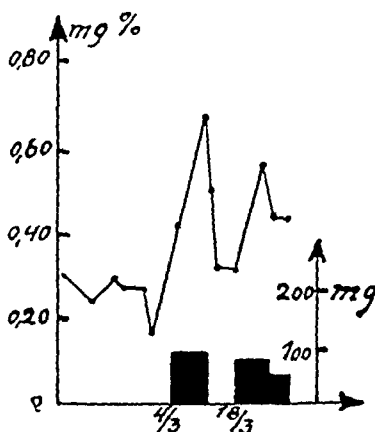
Case I.

No. 389/42. F. 40 years. Diagn. sciatica.

Adm. 24/11—41, disch. 31/3—42. Before the admission the patient has eaten plenty of fruit and vegetables daily.

24/11—4/3—42: Ord. plain diet — fruit.

4/3 —10/3	:	»	»	+ 1—2 oranges + apples daily.
11/3 —18/3	:	»	»	— fruit.
19/3 —25/3	:	»	»	+ 1—2 oranges + apples daily.
26/3 —30/3	:	»	»	+ 1 orange daily.

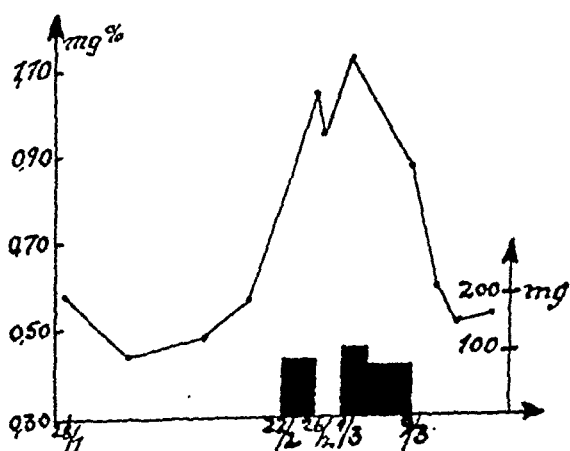


Conclusion: An evident increase is seen after a daily supplement of 1 or 2 oranges + apples.

Case II.

No. 355/42. M. 41 years. Diagn. sciatica.

Adm. 26/1—18/3—1942. Before admission the patient has eaten ordinary diet.



- 28/1—22/2: Ord. plain diet — fruit.
 23/2—25/2: » » + 6 apples daily.
 26/2— 1/3: » » — fruit.
 2/3— 3/3: » » + 2 oranges + 2 apples daily.
 4/3—10/3: » » + 4 apples daily.
 11/3—18/3: » » — fruit.

Conclusion: Rise after supplementary addition of 6 apples daily, further rise after 2 oranges, after that decrease in spite of daily addition of 4 apples; when the addition of fruit is stopped the patient reaches the initial values.

Case III.

No: M. R., M. 25 years. Medical man. Ordinary plain diet. Healthy.

9/4—21/4 42: 50 mg asc. acid daily + 1 orange daily.

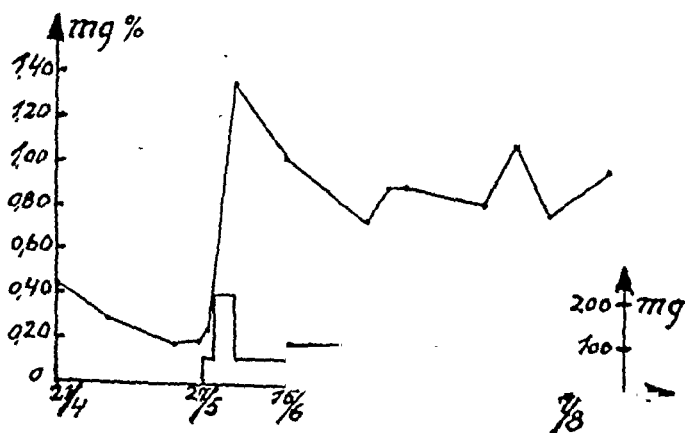
22/4—27/5 : Ord. plain diet — addition.

28/5—29/5 : » » + 50 mg asc. acid daily.

30/5— 3/6 : » » + 200 mg » » »

4/6—15/6 : » » + 50 mg » » »

16/6— 4/7 : » » + fruit.



5/7—8/7 42: Ord. plain + about $\frac{1}{2}$ kg strawberries daily.

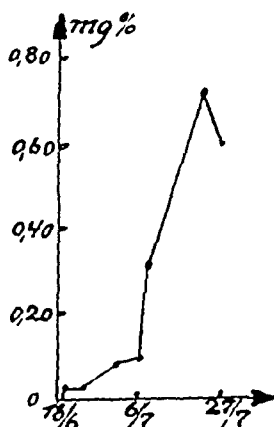
9/7—27/10 : » » — addition.

Conclusion: Fall during May on ordinary plain diet, steep rise after 200 mg ascorbic acid. Increase after addition of strawberries.

Case IV.

No. 90/42, F. 51 years. Diagn. sciatica.

Adm. 8/6—27/7—1942. Before the admission the patient has had ordinary plain diet.



18/5—27/7—42: Ord. plain diet — addition.

From about 4/7 the patient had new potatoes, a couple of times a plate of strawberries.

Conclusion: Distinct increase after new potatoes.

Case V.

No. 1130/42. M. 19 years. Diagn. rheum. fever. Acute nephritis.

Adm. 24/4—19/9—42.

24/4—2/7—42: Ord. plain diet — addition.

3/7—11/8 : » » + 50 mg asc. acid daily.

12/8—28/8 : » » — addition.

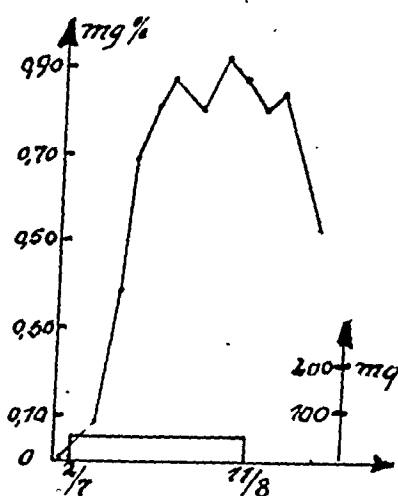
Conclusion: Distinct increase after 50 mg ascorbic acid, at the same time it must be taken into consideration that the patient has had new potatoes since 4/7. It appears that it is a fortnight before the patient reaches the values that correspond to the big daily supply.

Case VI.

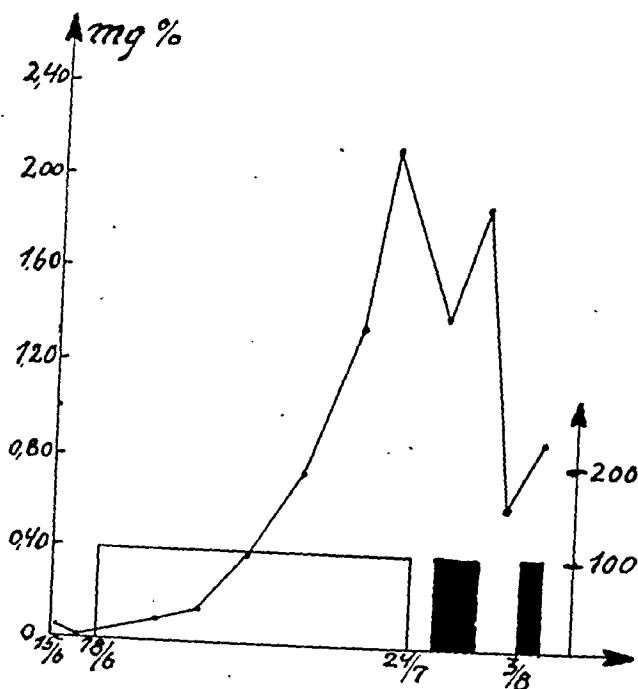
No. 889/42. M. 51 years. Diagn. sciatica.

Adm. 11/6—24/7—42. Before the admission the patient has eaten ordinary diet. After the discharge the patient came in for ambulatory treatment. In that way it was possible to continue the investigation until 7/8.

Case V



Case VI



- 11/6—19/6—42: Ord. plain diet — addition.
 20/6—24/7 : » » + 100 mg asc. acid daily.
 25/7—27/7 : » » diet — addition.
 28/7—31/7 : » » + fresh fruit daily.
 1/8— 3/8 : » » — addition.
 4/8— 7/8 : » » + fresh fruit daily.

Conclusion: Increase after 100 mg ascorbic acid daily, decrease after the addition was stopped. Distinct increase after fresh fruit. It is further seen that it is 12 days before the ascorbic acid rises materially in spite of the great daily addition.

The content of serum ascorbic acid on fairly constant diet.

In 45 patients the concentration of serum ascorbic acid has been determined 1 or 2 weeks after the admission and at the discharge 1 or 2 weeks later. During their whole stay in hospital the patients have had ordinary plain diet without supplement of vitamin C-containing foodstuffs. The investigations were undertaken during the months in which the composition of the hospital diet with regard to vitamins may be considered as fairly constant; thus patients who were admitted during the period July-August are not included here. In 42 of the patients about the same value of ascorbic acid content in serum was found in the two examinations. As an example a 54 yearold man suffering from sciatica may mentioned. 15 days after the admission the concentration of serum ascorbic acid was 0.32 mg per cent, 8 days later 0.29 mg per cent. In three patients only a fall in the concentration could be demonstrated, viz. 0.28—0.13 and 0.12 mg per cent. No explanation of this can be given.

Discussion.

It appears from the investigations that the content of serum ascorbic acid in one and the same individual adjusts itself to a relatively constant level, when the individual has eaten ordinary hospital diet — fruit for more than one week. Unfortunately our material is not large enough to elucidate the question whether different individuals on the same diet adjust themselves to the same level. The serum ascorbic acid level of normal persons is subject to considerable seasonal variations. The knowledge of the position of the average level and the size of the standard deviation in normal persons must be the basis of estimating whether the ascorbic acid concentration in this or that illness deviates from the normal condition, and it must be emphasized that the standard deviation in normal persons is extraordinarily large. It is unusual, however, to see spontaneous values above 1.5 mg per cent. With regard to the lower limit it has been shown by Crandom, Lund and Dill, that it can be 0.00 mg per cent for 4 months before the appearance of symptoms of scurvy. In the material presented here the content of serum ascorbic acid is 0.0 mg per cent in 25 persons

without any of these persons showing symptoms of scurvy. The zero values appear during the months of February to June. It depends to some extent on the season whether zero should be considered a normal value. In May and June it can hardly be called an abnormal value. It appears further from the material presented here, that there is a definite relationship between the ascorbic acid content of the diet and the content of serum ascorbic acid. If one considers for instance the curves of the last three patients it is obvious, that the concentration of serum ascorbic acid varies with the administration of fruit or pure ascorbic acid in such a way, that the more ascorbic acid is given the higher the level to which the concentration of ascorbic acid adjusts itself. When some authors have not found agreement between the ascorbic acid content of the diet and the serum ascorbic acid values, it is conceivable that they have not been aware of the long time (see patients V and VI), which passes before a patient with 0 mg per cent adjusts himself to a level that corresponds to the very great daily administration. Another factor which possibly asserts itself is the rapid fluctuations in the concentration of serum ascorbic acid after a single bigger dose if the initial value lies above zero (cf. Bræstrup and Lund).

Summary.

Farmer and Abt's method has been employed and elucidated in different ways. In 346 normal persons a distinct seasonal variation has been demonstrated by means of 650 analyses of serum ascorbic acid over a period of 16 months. A very large standard deviation of the concentration of serum ascorbic acid is found. Serum ascorbic acid 0 mg per cent is found in 25 patients without any of these showing symptoms of scurvy.

By additional administration of vitamin C to a few persons an increase in the concentration of serum ascorbic acid has always been demonstrated when the period of the experiment was sufficiently long. In 42 patients out of 45 on uniform hospital diet the ascorbic acid content of serum is found unchanged over a period of 2 to 4 weeks. From the present material the conclusion can be drawn that there is close relation between the ascorbic acid added through the mouth and the ascorbic acid content of

serum. The experimental material is not sufficiently large to elucidate the question whether a number of individuals on uniform diet will adjust themselves to the same serum ascorbic acid level.

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Some Investigations on the Ascorbic Acid Content in Serum from Patients Suffering from Peptic Ulcer.

II.

By

INGRID EBBESEN and MOGENS RASMUSSEN.

(Submitted for publication February 4, 1944).

In a previous paper we have investigated serum ascorbic acid in normal persons at Aarhus County Hospital. Simultaneously we had the opportunity to undertake ascorbic acid analyses in serum from patients with peptic ulcer, duodenal ulcer and gastro-duodenitis, which in what follows will be described as ulcer cases. The results are presented here. At the admission no difference could be demonstrated between the content of serum ascorbic acid in normal persons and in untreated ulcer cases. On the other hand a marked fall in the concentration of serum ascorbic acid could be demonstrated during the treatment of the patients on ulcer diet.

Platt (1936) has described 3 cases of scurvy in ulcer cases which had kept a one sided diet for some years.

By means of Harris and Ray's modification of Tillman's method of estimating the ascorbic acid excretion in urine several workers have believed that they could prove C avitaminosis in cases with peptic ulcer. Thus Lazarus (1937) in 15 cases with bleeding ulcer and in 3 cases with non-bleeding ulcer finds an ascorbic acid excretion in urine, that is considerably lowered compared with a normal material from 4 persons. Rivers and Carlson (1937) found lowered ascorbic acid content in plasma

from ulcer cases. By treating with 5 g ascorbic acid an obvious improvement in the patients' condition was established. Portnoy and Wilkinson (1938) found that 55 patients with ulcer disease «suffered from severe vitamin C deficiency». The plasma concentrations determined by Farmer and Abt's method are stated to lie from 0.60 to 1.85 mg per cent, in 50 normal persons, and from 0.14 to 0.59 mg per cent in ulcer patients. Bjorkman and Bachman (1939) demonstrate lower ascorbic acid values than normal in 27 cases with ulcer. In a later paper by Bachman (1939) it is found that patients with bleeding ulcer have lower ascorbic acid content in serum than normal patients on the same diet. In none of the works cited above ulcer cases showing symptoms of scurvy are mentioned. Rao (1938) found normal ascorbic acid content in blood from 15 patients with peptic ulcer. Trier (1940) arrived at the same result.

Own investigations.

The investigations were commenced in November 1941 and completed in April 1943. In September 1942 no analyses have been undertaken on account of the authors' illness. Farmer and Abt's method was employed. A detailed description of the method used is given in the above-mentioned paper on some investigations on serum ascorbic acid in normal persons.

The material consists of 63 patients, out of which ulcer in ventricle or duodenum had been demonstrated by X-rays in 52 cases. None of the 63 patients had had dietetic treatment immediately before the admission. Determinations of serum ascorbic acid were undertaken on all the patients at the admission into hospital. All results appear from fig. 1. The separate values are indicated by points. 8 cases were accompanied by hematemesis, these are indicated by circles. The fully drawn line indicates the arithmetic mean for the separate months. For comparison the seasonal variation curve found for normal persons by the authors is plotted (dotted line).

It is obvious that the average curve for ulcer cases shows the same seasonal variation as the curve for the normal material. The average curve for the ulcer cases lies now a little above and now a little below the normal values. Hematemesis does not seem to appear especially among the patients with low serum ascorbic acid. In the material presented here there is no basis for believing that the concentration of serum ascorbic acid in untreated cases is lower than in normal subjects.

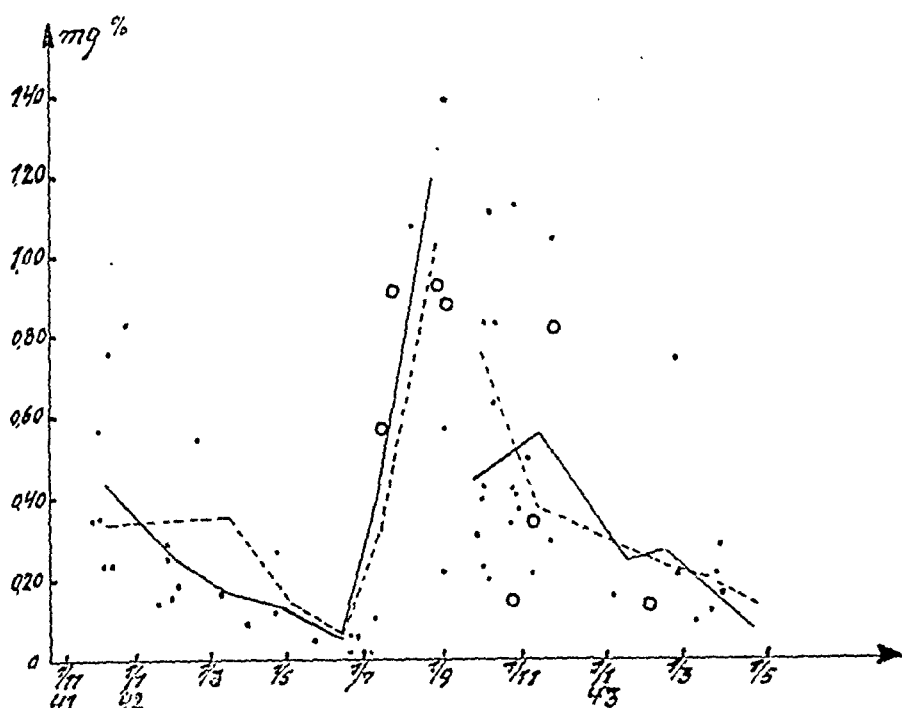


Fig. 1. Seasonal variation curves for ascorbic acid content in serum.

Abscissa: time.

Ordinate: mg. per cent ascorbic acid in serum.

Curve (—): monthly arithmetic means for 63 untreated ulcer cases.

Curve (---): variation curve for normal persons.

Points and circles indicate the separate ascorbic acid values for ulcer cases.

Circles indicate + hematemesis.

During their stay in hospital the patients were treated with the ordinary ulcer cure of the hospital, in which the diet consists of 1 l. of egg milk + $\frac{1}{2}$ l. of oatmeal soup for the first two days. During the following days it is supplemented with ricemeal porridge and semola gruel, later on tea and biscuits are added. During the following week there is a rise to a diet that may be indicated as fever diet, after which the diet is increased to a lenient diet in the course of one to two weeks. The cure thus lasts for about 4 weeks. In the cases where ulcer was complicated by hematemesis or melena without pain the cases were treated according to a somewhat different principle, purée diet being given before proceeding in 8 to 10 days to a lenient diet. In order to examine the influence of dietary treatment on the content of serum ascorbic acid the concentration was estimated at an interval of 3 to 6 days in 35 cases altogether. In fig. 2 the ascorbic acid concentrations in 5 of the cases have

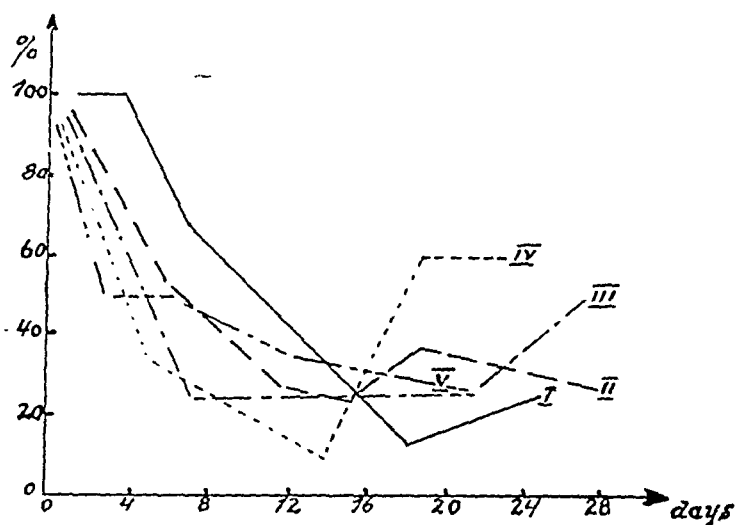


Fig. 2. Ascorbic acid content in serum during ulcer treatment in 5 cases.

Abscissa: time in days from beginning of treatment.

Ordinate: Ascorbic acid concentrations in per cent of initial values.

Curve I (—): J. J. No. 1552/42. Adm. 19/11 42. Disch. 12/12 42.

Curve II (---): J. K. " 1007/42. " 25/7 42. " 22/8 42.

Curve III (---): E. S. " 1385/42. " 13/10 42. " 1/11 42.

Curve IV (-·-·-): B. K. " 1328/42. " 7/10 42. " 31/10 42.

Curve V (·-·-·): S. N. " 1581/42. " 25/11 42. " 19/12 42.

been plotted. The cases are typical, but none of the other cases in the material differ essentially from these. The initial concentration is put arbitrarily at 100 per cent. It is seen from fig. 2, that the fall in concentration amounts to about 75 per cent during the first 12 to 16 days. When the patients get potatoes the ascorbic acid concentration rises.

It is well known that the ulcer diet is poor in vitamin C-containing foodstuffs. During the first two weeks the patients have vitamin C supplied exclusively through the milk, the ascorbic acid of which is given very varying; the patients cannot be supposed to have more than 10 to 15 mg ascorbic acid added through this foodstuff daily. According to Crandon, Lund and Dill (1941) the first symptoms of scurvy appeared when the ascorbic acid content in serum had been zero for three months. When 0 mg per cent is found it should be possible, according to what has been stated, to provoke scurvy when these patients are put on ulcer diet, and in this connection two cases will be mentioned in detail.

The first case was a 67 year-old man suffering from bleeding ulcer. Adm. from 10/6—6/7 42. The patient had been ill for about

one month. The ascorbic acid content in serum was 0.03 mg per cent at the admission. On account of cardialgy the patient was treated with ulcer diet. 17 days after admission circumpillar bleedings appeared, which disappeared by oral treatment with 150 mg ascorbic acid per day.

The other patient was a 67 year-old woman suffering from an obstructing callous ulcer, adm. to hospital from 19/5—25/7 42. The patient had for 9 months had pain in the epigastrium attended by violent vomiting after meals. The patient was treated dietetically for 20 days; but as she still vomited frequently an anterior gastro-enteroanastomia with enteroanastomia was made. The content of serum ascorbic acid was 0 mg per cent. After the operation the patient was exceedingly exhausted, circumpillar bleedings now appearing on the body and the extremities, suffusions on femora and crura and gingivitis. After treatment with ascorbic acid $\frac{1}{2}$ g intravenously per day the symptoms disappeared in the course of a fortnight. The patient was discharged on a lenient diet feeling completely well. It may be added that the patient at a later admission in April 1943 suffered from pellagra. In the first patient it is believed that the diagnosis beginning scurvy can be made, probably caused by dietetic treatment at a time when the Danish food is poorest in vitamin C. The development of scurvy in the other patient is presumably due to lowered ascorbic acid supply on account of the violent vomiting in connection with a diet poor in vitamin C. On the basis of what is stated above it is advisable during the months poor in vitamin C to supplement the diet with vitamin C.

Summary.

Determinations of ascorbic acid concentration have been undertaken at the admission on 63 untreated patients suffering from peptic ulcer and gastro-duodenitis. The concentrations are found to be within the normal limits or error. None of the patients showed symptoms of scurvy at the admission.

During dietetic treatment the ascorbic acid was estimated in 35 ulcer cases at intervals of 3 to 6 days. In all cases a considerable fall could be established. This fall no doubt may contribute to provoking scurvy and two cases are reported, in which scurvy seemed to develop in connection with the ulcer diet.

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Reexamination of Patients with Exophthalmic Goiter treated Conservatively.

By

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Within a few years the introduction of the preoperative iodine therapy, resulting in a considerable decrease in the operation mortality, has given surgery a dominating position in the treatment of exophthalmic goiter. Still, opinions are yet somewhat divergent as to the more precise establishment of the range of indications for the operative treatment, so that in the various hospitals a varying number of patients are still given conservative treatment, entirely medical or combined with X-ray treatment.

In recent years various authors have presented large materials showing the good results obtained with modern operative treatment. In this country, Windfeld (15) and Hertz (6) have given comprehensive reviews of the available literature on this subject together with their own experiences.

In his account of the material from the Dep. D (Surgery) of the Rigshospital in the period of 1926—35, and from the Surgical Dep. of the Finsen Institute in the period of 1929—35, a total of 395 patients, Windfeld (15) found an operation mortality of 2.8 %. On personal reexamination of 340 of these patients he found 90.6 % to be able to work, while 4.1 % showed a lowered capacity for work and 5.3 % were unable to work. Hertz (6) presented a material

of 94 patients from the Dep. C (Surgery) of the Rigshospital in which the operation mortality was 0. On reexamination of 90 of these patients 82 (91 %) were well.

Also the results of X-ray treatment have been analyzed in several recent works, some of them from this country.

Thus Raagaard (11) reexamined 68 out of 89 patients who had been treated in the Aalborg County Hospital in the period of 1922—35, and found 48 (70.6 %) recovered, 12 (17.7 %) improved and 5 (7.4 %) unchanged, while 1 (1.5 %) had died of exophthalmic goiter, and 2 had died from some other disease. Bjørneboe (1) reexamined 79 patients who had been given X-ray treatment in the Bispebjerg Hospital in the period of 1924—34 and found that 5 had died from diseases other than exophthalmic goiter and 3 were suffering from some other lesion. Of the remaining 71 patients 7 (9.8 %) died of exophthalmic goiter, 8 (11.3 %) were subsequently given operative treatment, 12 (16.9 %) were ill and 44 (62 %) were well.

In contrast hereto, more recently only relatively few studies have been reported on the course of exophthalmic goiter under entirely medical treatment, although such an account may be said to be prerequisite to the evaluation of the effectivity of other therapeutic methods.

On the whole, however, the therapeutic results presented in the various accounts are directly comparable but to a slighter extent. Even the available patient materials differ considerably, as the various investigators are widely divergent in their limitation of the clinical picture of exophthalmic goiter. Many of the older materials are largely made up of the more severe forms of the disease, while the more recent materials generally include a greater number of relatively mild cases, partly because hospitalization of such patients has become more common, partly because of improved diagnostic measures.

Furthermore, as a rule, the more recent materials of patients given medical treatment will represent merely a section of the total material of the department concerned, as a greater or smaller percentage of the patients have been submitted to surgical or radiological treatment. The range of indications for the various methods of treatment has been established after rather different principles. In some clinics, for instance, cardiac complications

have been looked upon as contraindicating operative treatment, whereas in other clinics the operative treatment is withheld only from patients with manifest cardiac insufficiency. Also the different social conditions of the patients have had a considerable influence on the choice of treatment.

In follow-up studies on such materials, moreover, several other conditions will make a comparison of the materials rather difficult: insufficient size of the material, varying lengths of the observation period, grouping of the cases after different principles and finally the unavoidable subjective judgment, which asserts itself particularly in a disease as exophthalmic goiter, in which the objective findings not infrequently are in striking conflict with the complaints of the patients.

Of course, the clinical employment of determination of the basal metabolic rate as a routine measure has contributed considerably to settle these aspects of the question.

For the above-mentioned reasons the accounts of the case mortality for medical treatment of exophthalmic goiter vary greatly in the older material, between very low values and values round 30 %. In his account of the total literature prior to 1901, Sattler (12) found a case mortality of about 11 %.

In a material from St. Bartholomew's Hospital, London, in the period of 1920—30, Fraser (4, 5) (1930—31) found the following results from conservative treatment of 64 patients: 3 died of intercurrent diseases; of the remaining 61 the results were good in 23 (37.7 %), poor in 15 (24.6 %), bad in 3 (4.9 %), and 20 (32.8 %) had died of exophthalmic goiter.

Engel (2) (1932) worked up the material from the Medical Clinic I of the Seraaphimer Hospital, Stockholm in the period of 1913—20, a total of 200 patients. Of 94 patients who were given entirely medical treatment 13 had died of intercurrent diseases, of the remaining 81 patients 45 (55.5 %) were capable of work, 8 (9.9 %) were partially able to work, 8 (9.9 %) were unable to work, and 20 (24.7 %) died of exophthalmic goiter.

Solling (14) (1916) worked up the patient material from the Medical Departments of the Royal Frederik's Hospital and the Rigshospital, Copenhagen, in the period of 1903—13. On reexamination of 75 out of 116 non-operated patients he found: 15 (20 %) quite well, 8 (10.6 %) considerably improved, 32 (42.6 %) unaffected.

ed but partly able to work, while 19 (25.3 %) had died of exophthalmic goiter (6 of them before their discharge from the hospital).

Finally, Fenger (3) (1928) worked up the material from the two medical departments of the Rigshospital in the period of 1913—24. On reexamination of 77 out of the 86 patients who had been given entirely medical treatment, he found 11 (14.3 %) recovered, 15 (19.5 %) nearly recovered, 11 (14.3 %) improved, 15 (19.5 %) unchanged, while 25 (32.4 %) died of exophthalmic goiter (17 of them before their discharge from the hospital).

The literature has brought but scanty accounts of the therapeutic results of systematic protracted iodine therapy. Eggert Møller (10) observed 25 patients under iodine treatment (75—200 mg daily) lasting from one month to 2 ½ years and found 1 patient getting worse, 1 recovered and the remaining all considerably improved.

Krarup & Schmidt (7) tried to carry through a protracted iodine therapy in 14 out of a total of 96 patients who in 1941—42 were admitted to the Med. Dep. B, the Bispebjerg Hospital. The results were characterized as good in 2 cases, fairly good in 5, and bad in 7; 5 of the latter had to be readmitted later on for operative treatment.

Writers' Studies.

In the summer of 1943 we followed up the exophthalmic goiter patients who in the period of 1931—40 inclusive had been under non-operative treatment in the three Medical Departments II, III and VII of the Kommune Hospital¹. During this period a total of 191 patients with exophthalmic goiter were admitted to the three departments. As will be evident from Table 1, the yearly number of patients was practically constant or perhaps increasing a little. In the following years, however, there was a very marked increase in the number of such patients — something which was observed also in other clinics (8) — as in 1941, '42 and '43 respectively 34, 84 and 120 patients were admitted. The principles of treatment have differed somewhat in the three departments, and in the period concerned they have varied somewhat also in the indi-

¹ We are greatly obliged to Dr. H. Heckscher and Dr. Tage Bjerling, Chief Physicians of Dep. II and Dep. VII, respectively, for permission to make use of the material from their departments.

vidual departments. In the first years a relatively great number of the patients were given entirely medical and radiological treatment, whereas the operative treatment was dominating in the later years.

Table 1.

Exophthalmic Goiter Patients admitted to the Three Medical Departments of the Kommune Hospital in the Period of 1931—40.

Year	Entirely medical treatment	X-ray treatment	Operative treatment	Total material
1931	7	5	3	15
1932	9	2	4	15
1933	9	4	4	17
1934	14	1	5 (+ 3)	20 (+ 3)
1935	13	1	7	21
1936	7 (+ 1)	1	4	12 (+ 1)
1937	11 (+ 1)	1	12 (+ 3)	24 (+ 4)
1938	7	1 (+ 1)	10	18 (+ 1)
1939	3 (+ 1)	1	19 (+ 1)	23 (+ 2)
1940	6	2	18 (+ 1)	26 (+ 1)
	86 (+ 3)	19 (+ 1)	86 (+ 8)	191 (+ 12)

The figures in parenthesis give the number of patients who previously had received another form of treatment.

Entirely medical treatment was given to 89 patients, 3 of whom had been submitted to operative treatment on previous admission within the period here concerned. 51 of the patients were treated with iodine, 38 merely with sedatives and confinement to bed. None of these patients were given X-ray treatment during their stay in the hospital (but 5 of them were given radiological treatment after their discharge).

In most of the iodine-treated cases the remedy consisted in various solutions of potassium iodide or iodine + potassium iodide. The doses employed varied greatly, from 40 to 300 mg iodine per day, being mostly about 150 mg. A small number of patients were given diiodotyrosin in doses varying from 200 to 600 mg per day. The duration of the iodine treatment varied between 2 and 9 weeks, but was most often about one month.

X-ray treatment was given to 20 patients, one of whom previously had been submitted to operative treatment. The irradiation was usually given on 3 fields, right and left lobe of the thyroid, besides the thymus (in series with a total surface dose of 1000—1800 r and single doses of 150—200 r). Four patients were given two series of X-ray application (total surface dose of 1150—4300 r); 4 patients were given three series (3000—4800 r); 3 patients received four series (6300—7800 r) and 1 patient received six series (a total of 7400 r).

After their discharge, as mentioned, X-ray treatment was given to 5 additional patients who in the hospital had received entirely medical treatment.

Operative treatment was given to 91 patients; in 8 of these cases, however, entirely medical treatment had been tried for a considerable length of time before the operation.

Table 2 gives a survey of the percental occurrence of the various symptoms on admission of the patients.

As in the further working-up of this material a particular account will be given of the patients who received X-ray treatment, all these patients have been grouped together in Table 2 even though 5 of them received X-ray treatment only after discharge from the hospital. From Table 2 it is evident that the group of patients treated operatively comprises more severe cases of exophthalmic goiter than do the groups given entirely medical treatment, as the values for the basal metabolism on admission to the hospital exceeded 140 % in 75 % of the operated patients, but only in 36 % of the patients treated medically. Also the group that was treated radiologically includes relatively many rather severe cases.

A total account of the symptoms in all the patients gives values which correspond very closely to the findings reported in the material from the Med. Dep. B of the Bispebjerg Hospital Copenhagen from about the same period (1932—41) (13).

It is reasonable to assume, then, that the patient material here examined constitutes a typical section of the exophthalmic goiter patients who in the period here concerned were under treatment in medical clinics in the city of Copenhagen, not resulting from any sort of selection of the patients at the time of admission.

Table 2.

Survey of the Occurrence of Various Symptoms in the Different Groups of Patients.
(Percental distribution.)

		Entirely medical treatment	X-ray treatment	Operative treatment	Total material	Material from the Bispe- bjerg Hosp., Dep. B, 13 1932-41
Sex	Women Men	87 13	76 24	85 15	85 15	93 7
Duration of illness in months	< 3 3-5 6-12 > 12 ?	14 18 25 39 4	12 24 28 28 8	15 12 26 36 11	14 15 27 37 7	20 18 22 37 3
Basal meta- bolic rate on admission	> 160% 140-160% 120-140% < 120% ?	10 26 51 9 4	16 44 36 4 0	26 49 21 4 0	18 39 36 6 1	17 45 33 4 1
Goiter	Large Medium Small Nodular None	8 25 43 6 18	8 40 36 0 16	19 57 19 0 5	13 42 31 2 12	8 45 25 2 20
Eye changes	Exophthalmos No ex., + other None ?	42 15 39 4	52 16 32 0	66 7 26 1	54 12 32 2	63 37 0
Pulse rate	> 120 100-120 80-100 < 80	2 19 60 19	0 36 48 16	3 34 52 11	2 28 55 15	3 29 55 12
Loss of weight	> 5 kg < 5 kg + 0 ?	33 4 26 12 25	32 4 20 12 32	40 4 20 15 21	36 4 23 13 24	55 29 — 9 7
Tremor	+ 0 ?	75 20 5	84 8 8	64 26 10	71 22 7	84 16 0
Average age (years)		40.8	38.6	37.1	38.8	38.8
Size of material		84	25	94	203	174

Table
Survey of

Group of patients	Total size of material	Died before discharge	Information lacking	Died of intercurrent disease	Reduced material	Died of exophthalmic goiter
	Number of patients in each					
I. Entirely medical treatm.	84	4	0	5	75	6 (8%)
II. X-ray treatment	25	4	1	0	20	0
Total material	109	8	1	5	95	6 (6.3%)
Windfeld's material 15	395	12	10	11	362	1 (0.3%)

The present follow-up studies comprised the non-operated patients, a total of 109, of whom 8 died before their discharge from the hospital.

Of the 101 discharged patients, 1 had gone abroad, so that any reexamination was out of the question for the present. 11 patients had died, and the cause of death could be ascertained in each instance. 31 patients had later on undergone operative treatment, and in each of these cases clinical data were available from the respective departments. Of the remaining 58 patients 50 turned up for reexamination. Concerning the remaining 8 patients, detailed data were obtained by mail from the patients themselves and from their physicians.

Thus information was obtained about 108 of the 109 not operated patients.

No information was collected about the 94 primarily operated patients, as this material will be worked up in the near future by another author. It may be mentioned here, however, that 6 of these patients died in connection with the operation — after 1935, however, only 1 out of 63 died in connection with the operation.

The material examined is divided into two groups.

Group I comprises 84 patients given entirely medical treatment.

3.

Total Material.

Operated later	Unable to work	Partly able to work	Able to work			
	Bad	Poor	Considerably improved	Recovered with rest symptoms	Fully recovered	Able to work, Total
Group I (% of reduced material)						
27 (36 %)	5 (6.7 %)	5 (6.7%)	10 (13.3%)	14 (18.6%)	8 (10.7%)	32 (42.6%)
4 (20 %)	3 (15 %)	4 (20%)	2 (10%)	3 (15%)	4 (20%)	9 (45%)
31 (32.6 %)	8 (8.4 %)	9 (9.5%)	12 (12.6%)	17 (17.8%)	12 (12.6%)	41 (43.2%)
2 (0.6 %)	19 (5.2 %)	17 (4.7%)	39 (10.8%)	104 (28.7%)	180 (49.7%)	323 (89.2%)

Group II comprises 25 patients given X-ray treatment; 20 of them received the radiological treatment during their stay in hospital, 5 after their discharge.

For tabulation of the therapeutic results (Table 3) the material is divided into sub-groups, corresponding to the classification employed by Windfeld (15), by which it becomes possible to compare the present material with his well-examined material of exophthalmic goiter patients given surgical treatment. In the account of Windfeld's material, for the sake of comparison, all the patients are included, both the ones who were reexamined personally and those who were followed up by mail.

In the classification of the therapeutic results, stress is laid upon the capacity of the patients for work, and an additional subdivision is made with a view to possibly remaining symptoms of thyrotoxicosis.

The designations here employed cover the following features.

- Fully recovered:* Full capacity for work. No complaints or objective symptoms. Basal metabolic rate and electrocardiogram normal.
- Recovered with rest symptoms:* Full capacity for work, normal rate of metabolism, but persistent subjective or objective symptoms.
- Considerably improved:* Full capacity for work. Basal metabolic rate slightly increased, also other remaining symptoms.
- Poor:* Working capacity somewhat decreased. Basal metabolic rate slightly increased; also other remaining symptoms.

E. *Bad*: Working capacity greatly reduced. Basal metabolic rate greatly increased; also other remaining symptoms.

Of the 84 patients given entirely *medical treatment* (Group I) 4 died before their discharge from the hospital (3 in thyrotoxic crisis, 1 with bronchopneumonia and cardiac insufficiency).

Information was obtained about all the patients discharged. 5 of them had died of intercurrent affections (hæmorrhagic diathesis; empyema; cholelithiasis; intestinal hæmorrhage; tuberculous pleurisy and peritonitis).

Accordingly, the reduced material then comprises 75 patients.

Of these 75 patients 6 (8 %) died $\frac{1}{4}$ to 6 years after their discharge, apparently as a result of their exophthalmic goiter. They all died with cardiac insufficiency. Positive data on auricular fibrillation were obtained in 5 of these cases.

27 patients (36 %) were subsequently submitted to subtotal thyroidectomy, on an average about 2 years (from 1 month to 7 years) after their discharge from the hospital. 4 patients were hospitalized again as early as 1 month after their discharge and were given surgical treatment in another clinic.

Of the remaining 42 patients 32 (42.6 %) were able to work. But only 8 of them (10.7 %) were perfectly free from symptoms, whereas 14 (18.6 %) had recovered — though with persisting rest symptoms — and 10 (13.3 %) were considerably improved.

5 patients (6.7 %) were only partially able to work; and 5 (6.7 %) were bad and completely unable to work.

Of the 25 patients given *radiological treatment* (Group II) 4 died before their discharge from the hospital (1 in thyrotoxic crisis, 1 with cardiac insufficiency, 2 of bronchopneumonia).

Information was obtained about 20 patients, as one had gone abroad.

4 patients (20 %) were later submitted to thyroidectomy, from 1 month to 6 years after their discharge from the medical department. Of the remaining 16 patients 9 patients (45 %) were able to work, but only 4 had recovered completely. 4 patients were partially able to work, and 3 were unable to work.

On comparison with Group I (entirely medical treatment) this small group presents no definitely demonstrable effect of the X-ray treatment. It is to be mentioned, however, that this material undoubtedly is a selected part of the non-operated patients

and includes relatively many rather severe cases of thyrotoxicosis. On this account and because of the small size of the material, then, no definite conclusions can be drawn as to the effect of the X-ray treatment given. Nor can any sure connection be made out between the intensity of the treatment and the subsequent course of the cases.

As a supplement to the total account recorded in Table 3, Table 4 gives a survey of the basal metabolic rate observed on reexamination or at an intervening readmission for operation. At the reexamination the basal metabolic rate was determined ambulatorily in the Laboratory of National Health Insurance Physicians. Unfortunately, data on the rate of metabolism are wanting in several cases, some of the patients living in the country at the time of the reexamination, while others could not be persuaded to submit to this examination.

Among the patients who had received entirely medical treatment, Group I, the basal metabolic rate was determined in 57 out of 69 patients. Unquestionably normal values (90—110 %) were found only in 14 (25 %). Decidedly increased values (over 120 %) were found in 32 patients (56 % of the examined).

Among the X-ray treated patients normal values for the basal metabolic rate were relatively more frequent (41 %), and values for decidedly increased metabolism less frequent (41 %) than in the preceding group.

Table 4.

Basal Metabolic Rate of the Patients on Reexamination or on Intervening Re-admission for Operative Treatment.

Group of patients	No. of patients	Basal metabolic rate (per cent of normal)							
		80—89	90—110	111—120	121—130	131—140	141—150	> 150	Not measured
		No. of patients in each group.							
I. Entirely medical treatm.....	69	3	14	8	6	7	5	14	12
II. X-ray treatm.	20	1	7	2	3	1	1	2	3
Total material	89	4	21	10	9	8	6	16	15

Table

Tabulation of the Material with Reference to the

Duration of illness at first admission	Group of patients	Total size of material	Died before discharge	Information lacking	Died of inter-current disease	Reduced material	Died of exophthalmic goiter	
< 1 year	I. Entirely med. treatm.	48	2	0	3	43	4 (9.3%)	
	II. X-ray treatment ..	15	1	1	0	13	0	
	Total material	63	3	1	3	56	4 (7.1%)	
> 1 year	I. Entirely med. treatm.	33	2—1	0	1	30	2 (6.7%)	
	II. X-ray treatment ..	8	2+1	0	0	6	0	
	Total material	41	4	0	1	36	2 (5.6%)	

Values under 90 % were obtained in 4 patients — 89 % in 2, and 84 % in 2, one of whom had been given X-ray treatment after the first examination. None of the patients showed any other sign of myxoedema.

Of the 84 patients given entirely medical treatment (Group I) 49 were given *iodine* while 35 received no *iodine*. Tabulation of the patients with a view to the two forms of treatment shows a pronounced concordance of the results. Nor was it to be expected that any definite information about the effectivity of the *iodine* treatment might be obtained in that way. For, as mentioned, most often this treatment was carried through only for a relatively short period, during the stay in the hospital, and only a few of the patients appeared to have continued this treatment after their discharge from the hospital.

Concerning a small group of 11 patients, definite information was obtained that systematic *iodine* treatment had been given as the only form of treatment through a considerable length of time (2—4 years). On reexamination 2 of these patients were found to have recovered completely, 3 were cured but with persistent symptoms, in 4 the condition was poor, in 1 it was bad (basal metabolic rate 151 %), and 1 died with cardiac

5.

Duration of Illness at the First Admission.

Operated later	Unable to work	Partly able to work	Able to work			
	Bad	Poor	Considerably improved	Recovered with rest symptoms	Fully recovered	Able to work. Total
group (% of reduced material)						
14 (32.5%)	3 (7.0%)	2 (4.7%)	7 (16.3%)	7 (16.3%)	6 (13.9%)	20 (46.5%)
2 (15.4%)	1 (7.7%)	3 (23.1%)	2 (15.4%)	2 (15.4%)	3 (23.1%)	7 (53.9%)
16 (28.6%)	4 (7.1%)	5 (8.9%)	9 (16.1%)	9 (16.1%)	9 (16.1%)	27 (48.3%)
13 (43.3%)	2 (6.7%)	3 (10.0%)	3 (10.0%)	5 (16.6%)	2 (6.7%)	10 (33.3%)
1 (16.6%)	2 (33.3%)	1 (16.6%)	0	1 (16.6%)	1 (16.6%)	2 (33.3%)
14 (38.9%)	4 (11.1%)	4 (11.1%)	3 (8.3%)	6 (16.6%)	3 (8.3%)	12 (33.3%)

insufficiency. In 1 patient auricular fibrillation, whereas this phenomenon had disappeared in 1; in 1 some changes had appeared in the terminal complex.

Thus the iodine treatment has not produced any better course of the disease in this group than in the material on the whole. But this does not allow of any conclusions as the group is very small and undoubtedly selected; for presumably the patients were given iodine treatment at home for the very reason that symptoms of thyrotoxicosis persisted.

In the hospital this small group did not differ distinctly from the material in general as to symptomatology. The level of the metabolism was under 120 % in 1, between 120 and 140 % in 6, and between 140 and 160 % in 4. At that time, however, in 6 (perhaps 7) of these patients the disease had already lasted for several years.

Information about the *duration of illness* before the first admission was obtained in altogether 104 of the 109 non-operated cases. The disease had lasted less than 1 year in 63, over 1 year in 41. In Tables 5 and 6 the cases are entered with a view to the duration of illness at the time of admission.

From Table 5 it will be noticed that the duration of illness prior to admission of the patients has only a relatively slight influence on the subsequent course of the disease.

Of the patients given entirely medical treatment (Group I) and in whom the lesion had lasted over 1 year, somewhat more were subsequently admitted to operative treatment (43.3 % as against 32.5 %), and fewer were able to work (33.3 % as against 46.5 %). 16.7 % were completely or partly unable to work (as against 11.7 %). The mortality figures show no definite difference (6.7 % as against 9.3 %).

On division of the material in smaller groups after the duration of illness (for instance < 3 months, 3—6 months, 6—12 months) the individual groups become too small to allow of any appraisal.

On tabulation of the material after the basal metabolic rate at the reexamination or intervening hospitalization for operation, decidedly increased values are found in 70 % of the patients

Table 6.

Basal Metabolic Rate of the Patients on Reexamination or on Intervening Re-admission for Operative Treatment. Tabulation with Reference to the Duration of Illness at the First Admission.

Duration of illness on first admission	Group of patients	No. of pls.	Basal metabolic rate, per cent of norm.							
			80—89	90—110	111—120	121—130	131—140	141—150	> 150	Not measured
			No. of patients in each group							
< 1 year	I. Entirely medical treatm. . .	39	3	8	5	2	5	3	6	7
	II. X-ray treatment	13	0	4	2	3	0	0	1	3
	Total material	52	3	12	7	5	5	3	7	10
> 1 year	I. Entirely medical treatm. . .	28	0	4	3	4	2	2	8	5
	II. X-ray treatment . .	6	1	3	0	0	0	1	1	0
	Total material	34	1	7	3	4	2	3	9	5

whose illness had lasted over 1 year at the time of their admission, but only in 50 % of the patients whose illness had lasted less than 1 year. On corresponding tabulation of the X-ray treated material alone, the groups become too small for any reliable estimation.

Division of the material after the *length of the observation period* appears not in any demonstrable degree to influence the distribution of the patients in the various categories of therapeutic results.

In Tables 7 and 8 the material is classified after the *basal metabolic rate* at the first admission of the patients.

From Table 7 it will be noticed that the lower the initial level of metabolism, the more favorable was the course of the disease in general. This difference is relatively slight, however, and it is to be mentioned that one half of the patients whose basal metabolic rate on the first admission was under 120 % subsequently were given operative treatment on account of persistent signs of thyrotoxicosis. A separate tabulation of the patients whose initial basal metabolic rate was over 160 % gives too small a material to allow of any definite conclusions. On the whole, however, there appears to be no difference in the course of the disease for this group and for the group with a basal metabolic rate between 140 and 160 %.

Turning to the *cardiac changes* in these patients, the following findings were recorded.

On admission, electrocardiography was performed on 72 of the 109 patients. At the reexamination or on intermediate readmission for operation data were available on the electrocardiographic findings in 58 patients and on auscultation of the heart on all the living patients and most of the dead.

Among the 84 patients who were given *entirely medical treatment* 10 presented *auricular fibrillation* already during their stay in the hospital.

This phenomenon occurred especially in the somewhat elderly patients, the average age of these 10 patients being 52.5 years as against 40.8 years for the entire group. The appearance of this phenomenon was found not to depend on the duration of illness, as this was less than 1 year for one half of the 10 patients — just as for the entire material.

Of these patients with auricular fibrillation 3 died in the hospital, and 3 died respectively $\frac{1}{2}$, 4 and 5 years after their discharge.

Table
Tabulation of the Material with Reference to

Basal metabolic rate at first admission	Group of patients	Total size of material	Died before discharge	Information lacking	Died of inter- current disease	Reduced material	Died of exophthalmic goiter	
>140%	I. Entirely med. treatm.	30	2	0	2	26	4 (15.4%)	
	II. X-ray treat- ment	15	4	1	0	10	0	
	Total material	45	6	1	2	36	4 (11.1%)	
120—140%	I. Entirely med. treatm.	43	1	0	3	39	2 (5.1%)	
	II. X-ray treat- ment	9	0	0	0	9	0	
	Total material	52	1	0	3	48	2 (4.2%)	
< 120	I. Entirely med. treatm.	8	0	0	0	8	0	
	II. X-ray treat- ment	1	0	0	0	1	0	
	Total material	9	0	0	0	9	0	

In 3 patients the auricular fibrillation was present on readmission for operation respectively 1 month, 15 months and 2 years after their discharge. In 1 patient, in whom auricular fibrillation was demonstrated during his stay in the hospital, this phenomenon had disappeared at the time of the reexamination, 4 years later, although the basal metabolic rate still was 125 %.

In 4 patients auricular fibrillation appeared after their discharge from the hospital. 2 of them died with signs of cardiac insufficiency respectively 3 months and 5 years after their stay in the hospital, where no auricular fibrillation had been demonstrated, but respectively bundle branch block and abnormal T waves. In the remaining 2 patients auricular fibrillation was found at the reexamination, 10 years after their discharge. In 1 of these

7.

the Basal Metabolic Rate at the First Admission.

Operated later	Unable to work	Partly able to work	Able to work			
	Bad	Poor	Considerably improved	Recovered with rest symptoms	Fully recovered	Able to work Total
(% of reduced material)						
11 (42.3%)	2 (7.7%)	1 (3.8%)	5 (19.3%)	2 (7.7%)	1 (3.8%)	8 (30.8%)
3 (30%)	1 (10%)	2 (20%)	0	3 (30%)	1 (10%)	4 (40%)
14 (38.9%)	3 (8.3%)	3 (8.3%)	5 (13.9%)	5 (13.9%)	2 (5.5%)	12 (33.3%)
11 (28.2%)	3 (7.7%)	4 (10.3%)	3 (7.7%)	10 (25.6%)	6 (14.4%)	19 (48.7%)
1 (11.1%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	0	2 (22.2%)	4 (45.5%)
12 (25.0%)	5 (10.4%)	6 (12.5%)	5 (10.4%)	10 (20.8%)	8 (16.7%)	23 (47.9%)
4 (50%)	0	0	1 (12.5%)	2 (25%)	1 (12.5%)	4 (50%)
0	0	0	0	0	1	1
4 (44.5%)	0	0	1 (11.1%)	2 (22.2%)	2 (22.5%)	5 (54.5%)

patients premature contractions had been found during the stay in the hospital, while the other at that time showed normal findings.

Of other electrocardiographic changes, *isolated abnormalities of the T waves* were found in 6 patients, 1 of whom died in the hospital, while another died 5 years later with auricular fibrillation and cardiac insufficiency. In 3 of these patients the abnormal changes had disappeared at the time of the reexamination, while they persisted in 1 (7 years after discharge from the hospital). On the other hand, this phenomenon had appeared in 3 additional patients at the time of the reexamination.

Bundle branch block was found only in 1 patient, who died 3 months later with auricular fibrillation. *Multiple ventricular premature contractions* were found in 1 patient who died 1 year

Table 8.

Basal Metabolic Rate of the Patients on Reexamination or on Intervening Re-admission for Operative Treatment. Tabulation with Reference to Basal Metabolic Rate on First Admission.

Basal metabolic rate on first admission	Group of patients	No. of pts.	Basal metabolic Rate, per cent of normal.							
			80—89	90—110	111—120	121—130	131—140	141—150	✓ 150	Not measured
			No. of patients in each group							
> 140%	I. Entirely med. treatment ..	22	0	5	2	2	3	1	6	3
	II. X-ray treat.	10	1	3	0	3	0	1	1	1
	Total material	32	1	8	2	5	3	2	7	4
120—140%	I. Entirely med. treatment ..	37	3	7	4	2	3	4	6	8
	II. X-ray treatm.	9	0	3	2	0	1	0	1	2
	Total material	46	3	10	6	2	4	4	7	10
< 120%	I. Entirely med. treatment .	8	0	2	2	2	0	0	1	1
	II. X-ray treatm.	1	0	1	0	0	0	0	0	0
	Total material	9	0	3	2	2	0	0	1	1

later with cardiac insufficiency. Premature ventricular contractions were found in 1 patient, who on reexamination — 8 years later — presented auricular fibrillation. The reexamination revealed the presence of a *nodal rhythm* in 1 patient who in the hospital had given a normal electrocardiogram.

Thus the mentioned electrocardiographic changes were found in altogether 25 of the patients. Auricular fibrillation occurred in altogether 14 (16.7 %) and in 4 of these cases it developed after their discharge.

Among the 25 *X-ray-treated* patients auricular fibrillation was found in 5 during their stay in the hospital. 2 of these patients died in the hospital, 2 presented auricular fibrillation also on reexamination — respectively 4 and 10 years later — and 1 showed a normal sinus rhythm on readmission for operation 3 years later, although the basal metabolic rate at this point of time was 170 %.

In addition, the reexamination revealed auricular fibrillation in 1 patient who in the hospital had presented a normal electrocardiogram.

Isolated abnormality of the T waves was found in 1 patient both on admission to the hospital and on readmission.

Finally the reexamination showed low voltage in 2 patients, 1 of whom had given a normal electrocardiogram on admission 12 years previously, while the other had not been examined electrocardiographically before. Both of these patients gave normal values for the basal metabolism at the reexamination.

Among the 94 primarily operated patients, 80 of whom were electrocardiographed, auricular fibrillation was found in 5 (1 of these has been mentioned before under Group I) and isolated changes in the T waves in 6.

So, in the *total material* of 191 patients 19 (10 %) had auricular fibrillation during their stay in the hospital.

Discussion.

A comparison between the therapeutic results of entirely medical treatment of patients with exophthalmic goiter (Group I) as ascertained here and the results of surgical treatment as presented by Windfeld's (15) material shows indisputably the superiority of the surgical treatment (Table 3).

After the medical treatment a lesser number of the patients became able to work (42.6 % as against 89.2 %), and only a few could be characterized as fully recovered, without any remaining symptoms (10.7 % as against 49.7 %). A considerably greater number presented more or less inconveniencing rest symptoms. In 13.4 % of the patients the state of health at the reexamination had to be characterized as »bad» or »poor» (as against 9.9 %).

Furthermore, no less than about one third (36 %) of the patients given medical treatment later — on an average 2 years after their discharge — had to submit to operative treatment on account of persistent symptoms of thyrotoxicosis.

8 % of the patients died of their illness (as against 0.3 %).

So the state of the patients at the reexamination was considerably worse after the medical treatment than after the surgical.

In addition, it is to be emphasized strongly that, in contrast to the surgical treatment, the medical implies a very considerable increase in the duration of the illness and disablement of the patients for a much longer period. This protraction of the illness will further increase the possibility of the development of complications, especially on the part of the heart.

The 10 patients whose condition at the reexamination was characterized as poor or bad, had been ill for 3—11 years, on an average 8 years, after the first admission to the hospital. In 1 of them auricular fibrillation had made its appearance. 3 had applied for disablement benefit, which had been granted them.

To the 27 patients who later were given surgical treatment, the interval between their first admission to hospital and the operation was on an average 2 years — at the most 7 years — of illness as far as may be judged from the case records, at least 17 of these 27 patients were operable at their discharge from the hospital.

As mentioned above, 8 (10.7 %) of the patients were fully recovered after the medical treatment (without X-ray treatment). In 7 of these cases the disease was relatively mild. In 6 of them the disease had lasted less than 1 year. From Tables 5—8 it is evident indeed that the therapeutic results are a little better for those patients who on admission had been ill for less than 1 year and in whom the basal metabolic rate was less than 140 %. The difference from the results for the other groups is only slight, however, and on the whole it will hardly be practicable a priori to make an individual prognosis with any particular degree of certainty.

In the material here presented the X-ray treatment appears not to have had any essential influence on the course of the disease in comparison to the entirely medical treatment. But the number of patients given radiological treatment is all too small to allow of any far-reaching conclusions.

With reference to the entirely medical treatment of exophthalmic goiter the conclusion of the follow-up studies here presented will be that we have to subscribe to the statement made by Means & Richardson (8): „... it is going to necessitate the expenditure of many months on an incomplete and uncertain cure, when symptomatic recovery is possible in a few weeks and with a high degree of safety by subtotal thyroidectomy in an iodine remission».

Summary.

In the period of 1931—40 altogether 191 patients were admitted for exophthalmic goiter to the three medical departments of the Kommune Hospital, Copenhagen. Of these patients 84 were given entirely medical treatment.

Of these 84 patients 4 died in the hospital, 5 died of intercurrent diseases after their discharge from the hospital.

The remaining 75 patients were reexamined in the summer of 1943.

32 (42.6 %) of the patients stated that they were able to work, although only 8 of them (10,7 %) were entirely symptom-free, while the remainder presented more or less pronounced rest symptoms.

5 (6.7 %) were partially unable to work, 5 (6.7 %) were entirely unable to work. 27 (36 %) had undergone operative treatment in the intervening period on account of persistent symptoms, and 6 (8 %) had died from exophthalmic goiter.

Data on the basal metabolic rate at the time of the reexamination or the intervening operation were available in 57 of the 69 surviving patients. An unquestionable increase in the rate of metabolism (over 120 %) was found in 32 (56 %). Values over 140 % were found in 19 (33 %).

Comparison with the results of operative treatment (Windfeld's material (15)) shows indisputably the advantages of surgical treatment.

The results of the medical treatment are best for the group of patients in whom the illness had lasted less than one year and whose basal metabolic rate kept at a level under 140 %. For the other groups of patients the therapeutic results differed but little, and on the whole it will hardly be possible to make any individual prognosis.

On reexamination of 25 patients given X-ray treatment no definite difference could be found between the therapeutic results obtained for this group and the results of entirely medical treatment.

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Sedimentation Rate and Room Temperature.

By

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In spite of the great part played by sedimentation reaction in the clinic for determining the state of health of patients and for establishing diagnosis, the inaccuracies attaching to this reaction have so far not been adequately probed. In the present paper it is proposed to discuss what is commonly looked upon as the most important source of inaccuracies, viz. the influence of the temperature.

Fåhræus (1921) was the first to deal at greater length with the conditions surrounding the sedimentation of the blood, even though, as he himself points out, various earlier authors had made observations of interest. That the reaction has great clinical usefulness was eventually shown above all by researches on the part of Westergren and other authors.

Fåhræus tried to ascertain how a previous heating, especially at a temperature exceeding 30° C, affects the suspension stability of the blood, but does not discuss the importance of temperature to the sedimentation itself.

Westergren (1924) gave definite directions to the effect that the reaction should be performed under »room temperature», i.e. 18° C., and this opinion is shared by a number of authors for instance Christensen and Holbøll (1937), v. Domarus (1937), Forster (1935), de Haan (1918), Johnson (1939), Katz (1922),

Löwenberg (1924), Noltze (1921), Reichel (1936), Rimini (1934) and Walton (1933).

Other values for standard temperature have, however, also been proposed, as for instance by:

<i>Author</i>	<i>Temperature</i>
Coorey (1925)	»cool room in winter, ice box in summer»
Raponsky (1934)	18°—25° C.
Beckmann (1938)	20° C. (Water bath)
Yardumian (1937)	20°—30° C.
Gordon and Cohn (1928)	23° C.
Sato and Otuka (1941)	37° C. (Thermostat)
Sasano and Ordway (1936)	37.5° C.

Of course changes of temperature inevitably occur in a room where the sedimentation test is performed. Furthermore, the room temperature during high summer in Sweden does not infrequently exceed 18° C., whilst considerably lower figures will often be registered in the winter. We can therefore quite understand Sato and Otuka (1941) when they say: »In einer Gegend wie in Formosa wird es am zweckmässigsten sein, einer Thermostat mit 37° C. zu gebrauchen», even though objections can be raised to the standard temperature suggested by them.

The sedimentation reaction usually increases with rising temperature. In some cases, however, it may happen that a lower figure is obtained at a higher temperature. Carlinfanti and Balestieri (1938) call this phenomenon »reazione paradossa» and Decker (1939) has employed the term »positive kalte SR». Sato and Otuka (1941) state that »die Skg. ist total (partiell) umgekehrt». By »total umgekehrt» is meant that the SR-curve shows lower figures at a higher temperature in the whole of its course, and by »partiell umgekehrt» in only a part of it. (Readings made after 15, 30, 60, 90 and 120 minutes.)

The following authors speak of SR as deviating from the rule, or as »positive kalte SR»; Carlinfanti and Balestieri (1938), Decker (1939 and 1941), Jessen and Bing (1940), Kubo (1925), Leffkowitz (1933), Löwenberg (1924), Maia (1930), Miyagawa (1939), Nagasako (1938), Sato and Otuka (1941), Stöcklin (1926) and Thygesen (1942). It is noteworthy that Reichel (1936) in his mono-

Author	Temp. in C investigated	Particulars number and type of patients examined	Result
Löwenberg (1924)	8° 18° 37°	neurologic-psychiatric	Progressive paralysis patients show »positive kalte SR». Epileptics and dementia paralytici conform to rule.
Stöcklin (1926)	1° 22° 37°	—	SR highest at 1° and lowest at 37°.
Henderson (1929)	8—22.5° 35—37°	Total of 90 cases, 59 leprosy, 31 kala-azar	In 30 cases SR higher at 8—22.5° than at 35—37°, in 2 cases equal figures, the rest according to the rule.
Maia (1930)	12° 24°	Total of 104 SR	2 SR show higher figures at 12°, 2 SR show equal figures, rest follow the rule.
Carlinfantini and Balestieri (1938)	3—4° 18—20° 37°	About 40 SR in all	«Bei einigen Blutproben mit gesteigerter Sgk kann in Bezug auf die Temperatur ein anomales Verhalten festgestellt werden, und zwar die höchste Verlangsamung bei Raumtemperatur und eine Beschleunigung bei jeder Temperatur-Veränderung sowohl nach 0° als nach 37°.»
Decker (1939)	3—4° 19—20°	3000 SR from 884 cases	106 out of 264 tbc cases, and 12 out of 13 acute scarlatina cases show »positive kalte SR».
Jessen and Bing (1940)	19° 40°	—	1 case of Morbus Banti: 106—55 mm 1 case of Uremi: 114—67 mm at 19° and 40° respectively.
Sato and Otuka (1941)	15° 30° 37°	Total 400 SR	10 % »partiell umgekehrt» 12 % »total » » 78 % »nicht » »

graph on Sedimentation Reaction makes no mention of this type of SR.

Results of investigations into SR contrary to the rule are given in the table on page 537. (The works by Kubo, Nagasako and Miyagawa have not been accessible to the present author.)

v. Neergaard (1923) says »dass die Temperaturabhängigkeit von Fall zu Fall eine so verschiedene ist, dass die Aufstellung eines allgemeinen Temperaturkoeffizienten vorläufig unmöglich ist».

Summing up it may be stated that previous authors have shown that temperature has a tendency to affect the result of sedimentation reactions, in that as a rule the rapidity with which sedimentation proceeds tends to rise with the temperature, which may directly influence the size of the standard error and also may produce differences of systematic character between materials; only exceptionally, sedimentation will proceed more slowly at higher temperature than at lower. No more detailed study is, however, available on this subject. The object of the present work is:

- 1) To determine whether the inaccuracies change with temperature so that, the standard error at high temperature is different from that at low temperature;

- 2) To try to find a measure for the average deviation in the rapidity of sedimentation caused by changes in temperature, most conveniently expressed in mm per degree (Celsius), and finally;

- 3) To examine whether a reversal in the rapidity of sedimentation at rising temperature (slower sedimentation at higher temperature) occurs to the extent of warranting the assertion that such reversals are not the result of random errors.

Investigation into error of measurement.

Westergren (1934) determined the error in sedimentation reaction by his method to be 10 % plus 1 mm (»etwa 10 % der Grösse des Ausschlags plus 1 mm«). This statement is based upon 280 double determinations, the per cent distribution of which is given for groups with sedimentation reactions of varying degrees. As, however, the absolute number in these groups is not given it is not possible to carry out an exact calculation of the standard error. We can estimate, however, that the standard error keeps well be-

low 10 per cent, approximately at 7 per cent. It is further more clear that the standard error can never be smaller than the reading error produced by reading to within 1 mm, which means that the standard error in any one case cannot be expected to drop below half a mm. These statements apply to findings at about 18° Celsius. Slight changes of temperature are one of the causes of this error of measurement, but there are also other factors in operation such as the more or less exact dilution of blood by adding a solution of citrate, reading errors which are more or less unavoidable etc. Since temperature is an important factor it is obvious that under otherwise equal circumstances the error of method must be greater when determinations are made under more varying temperature than under more uniform temperature. It is also desirable that the error of method should be determined at several different levels of temperature, so as to ascertain whether this is of any consequence in this connection. The author has carried out such determinations within the limits of room temperature.

The tests forming the basis of the present investigation were made during the summer of 1941 at the Eira Hospital, Stockholm, on dermato-venereal cases.¹

Table 1 a.

The error of measurement in sedimentation rate at 12°, 18° and 25° C. respectively. Men and women. Right and left arm.

Temperature	Sedimentation rate- group in mm					
	5—14		15—24		25—64	
	Number = 20		Number = 21		Number = 29	
	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours
12°	±0.69	±1.62	±2.52	±4.43	±4.38	±4.34
18°	±0.58	±1.10	±2.47	±4.54	±3.77	±4.62
25°	±0.51	±1.51	±3.15	±6.72	±4.69	±5.07
Mean standard error	±0.59	±1.41	±2.71	±5.23	±4.26	±4.68

¹ The author takes this opportunity of extending his cordial thanks to Dr. Gunno Willners, Head physician of the hospital, for his kind permission to make use of the material.

Table 1 b.

The error of measurement in sedimentation rate in mm. and per cent at 12°, 18° and 25° C respectively.
Men and women. Right and left arm.

Tempera- ture in C.	Sedimenta- tion rate in mm (Mean)	Error of measurement	
		mm	%
5—14 mm. Number = 20			
12°	7.5	0.69	9.2
18°	8.7	0.58	6.7
25°	12.7	0.51	4.0
15—24 mm. Number = 21:			
12°	17.1	2.52	14.7
18°	19.7	2.47	12.5
25°	27.6	3.15	11.4
25—64 mm. Number = 29:			
12°	41.6	4.38	10.5
18°	44.1	3.77	8.5
25°	56.6	4.69	8.3

Method used. The tests were made with two of Westergren's standardized 5 cm³ syringe (citrate/blood = $\frac{1}{4}$) in a cubital vein one test from the right arm and one test from the left. After removing the tip the sample was sprayed into two tubes. After stirring, two samples were pipetted from each of these tubes, and put aside for sedimenting according to Westergren's technique, within half an hour after performing the test, each at temperatures of 12°, 18°, and 25° C. Changes in temperature $\pm 0.5^\circ$ C. The thermostats were autogulated, furnished with glass doors, that is to say lightened. (Cp. Lenzi, 1934). At 12° the thermostat was furnished with frigid aggregate. The one at 18° was water-mantled and cooled with running pipe water and also heated by an electric element, whilst that at 25° was water-mantled and heated in the same way. Readings were made after 1 as well as after 2 hours. Usually two series were put aside for sedimenting simultaneously.

The tests were made by the author and were also handled and read by him or under his control. (Cp. Romanus, 1941).

The standard error of the method was consequently obtained by independent double determinations, i.e. one from the right and one from the left arm of the same patient.

Table 2.

The error of measurement in mm and per cent after one hour and two hours
Men and women at 12°, 18° and 25°.

Sedimenta- tion rate group in mm	Mean of sedimenta- tion rate in mm.	Error of measurement	
		mm	%
After 1 hour:			
5—14	9.1	0.59	6.5
15—24	20.8	2.71	13.0
25—64	45.8	4.26	9.3
After 2 hours:			
5—14	24.2	1.41	5.8
15—24	47.2	5.23	11.1
25—64	63.2	4.68	7.4

The standard error of the method is given in Table 1 a in mm. In Table 1 b the same error is given for different rates of sedimentation and is also given as a percentage. This error was obtained from differences between the first test (the second test from each arm only served for checking the mixture) of the right and left arm respectively at each of the temperatures 12°, 18° and 25°. As there was no marked difference between these groups the figures for the different temperatures were fused. These will be seen from table 2.

We find further that the standard error expressed in millimetres both for one hour's and two hours' rates increases with a rising sedimentation rate (excepting the 2-hours' rates in the groups 15—24 and 25—64 where the standard errors are about equal). This is after all reasonable. When the sedimentation is higher there is more scope for variation. If, on the other hand, the standard error is calculated in percentages we find on the whole the same rates, although they are slightly smaller in the groups 5—14 and 25—64 for 1 hour's as well as for 2-hours' rates.

The percentage standard error is on the whole smaller than the figure given by Westergren and is smallest for the group 5—14 mm.

The investigation has thus shown that the effect of tempera-

Table 3.

Deviations in sedimentation rate in mm per 1 hour from 12° to 18°, 18° to 25° and 12° to 25°. Men and women.

Sedimentation rate-group in mm	Number of cases = N	Deviation in sedimentation rate in mm	Number of cases outside the limits of the error of measurement.			
		$M \pm \epsilon(M)$	Upwards	Downwards	Total	% of N
Deviation from 12°—18°:						
5—14	20	1.2 ± 0.26	2	—	2	10.0
15—24	22	2.1 ± 0.59	—	—	—	—
25—64	31	2.2 ± 1.1	1	—	1	3.2
Deviation from 18°—25°:						
5—14	20	3.8 ± 0.33	17	—	17	85.0
15—24	22	7.1 ± 0.44	1	—	1	4.5
25—64	31	12.4 ± 1.0	4	—	4	1.9
Deviation from 12°—25°:						
5—14	20	5.0 ± 0.47	20	—	20	100
15—24	22	9.1 ± 0.78	6	—	6	27.3
25—64	31	14.6 ± 1.9	9	1	10	32.3

ture on the standard error is fairly small (cp. Table 1) under the variation in the temperature that occur when the reaction is carried out in room temperature in the ordinary way.

The variations of sedimentation rates with room temperature.

An investigation into the sedimentation at rising temperature shows that sedimentation rises with the temperature as well after 1 hour as after 2 hours. The average differences are as a rule significant if we put the values for the right and left arms together. This applies in about equal degree both to men and women, for which reason the data may be safely amalgamated, as was also done in Table 3. This table also gives the result of an inquiry into the number of cases that can be said to lie outside the limits of the previously calculated error of method. In other words, it is possible to decide if the changes of temperature produced

Table 4.

Deviation in sedimentation rate, calculated per degree, in Rimini's material and the material of the present author.

Sedimentation rate group in mm/1 hour	Temperature	Total deviation in mm	Difference in total deviation in mm	Calculated deviation per degree in mm
Author's material:				
5—14	12—18°	1.2±0.26	} 2.6±0.41 (2.1±0.41) ¹	0.21±0.043
	18—25°	3.8±0.33		0.54±0.047
15—24	12—18°	2.1±0.59	} 5.0±0.73 (4.0±0.73) ¹	0.35±0.098
	18—25°	7.1±0.44		1.01±0.063
25—64	12—18°	2.1±1.1	} 10.2±1.49 (8.4±1.49) ¹	0.35±0.19
	18—25°	12.4±1.0		1.77±0.14
Rimini's material:				
25—64	10—15°	8.0±0.57	} 3.6±0.88	1.59±0.11
	15—20°	4.4±0.34		0.87±0.068
	20—25°	7.0±0.49	} 2.6±0.60	1.40±0.098
	25—30°	5.5±0.66		1.09±0.13

upward and downward deviations of such a size that they could not in the individual cases be attributed to the error of the method used. When passing on from 12 to 18° we encounter isolated deviations upwards and downwards, but these are so few that it is doubtful whether they are real. It should be borne in mind that the calculated errors of method are based upon very limited material and can therefore be slightly too small. If based on greater material it is quite conceivable that the errors could prove a little larger or smaller. When passing from 18 to 25° upward deviations are quite common whereas evidence of any downward deviations is lacking. In the passage from 12 to 25° upward deviations are commoner still whilst only one definite downward deviation is met with.

We conclude therefore in the first place that when the tempe-

¹ Difference between the calculated changes from 12° to 18° and from 18° to 24°.

perature rises from 18 to 25° deviations outside the limits of the error of method will occur more commonly than when the temperature rises from 12 to 18°. We further find that within the group of 5—14 mm deviations outside the limits of the error of method are more common at the higher temperature than at a lower, the difference in frequency between these two temperatures (18—25°) amounting to 75 ± 10.4 per cent; whereas in the group of 25—64 mm the difference in frequency at the same change of temperature is 9.7 ± 6.7 per cent. In other words a difference could be definitely established only in the SR-group of 5—14 mm.

In order to ascertain if there is any increase in the sedimentation rate from 12—18° and 18—25° a further table — No. 4 — has been drawn up. So as to get comparable figures a calculation has been made by interpolation to obtain the increase per degree. This is found to be greater from 18—24° than from 12—24°. The difference is statistically significant. The calculated increase per degree holds of course good only subject to the change being uniform. By way of control the author has made a calculation on Riminis' (1934) material. This reveals that the increase from 15—20° is smaller than the increase from 20—25°, which conforms to the facts established by the author. From 10—25°, however, the increase is greater than for 15—20°. This might possibly indicate that at 10—11° there is a critical temperature area. The material is, however, too small to permit of positive conclusions being drawn.

The occurrence of SR against the rule.

During the period of January to May 1939 routine double tests on SR were carried out in the medical ward of the county and town hospital of Hälsingborg.¹

Method of procedure. Tests were made with a Westergren standard syringe of 2.5 cm³ as with the usual routine tests, partly on newly arrived patients and partly on the regular weekly series. After mixing the blood fluid in the syringe this was emptied in equal parts into two tubes. After stirring the blood fluid was pipetted into the respective tubes within 1 hour after completion of the tests. The tests were pipetted at the central laboratory at a temperature of about 21° C with a maximum difference of

¹ The author wishes to offer his respectful thanks to Th. Stenström M. D., the chief of the hospital, for permission to make use of this material.

Table 5.

Deviations in sedimentation rate outside the limits of the error of method from 11° to 21° and from 15° to 21° in the Hälsingborg material.

Sedimentation rate-group in mm	Investigation of method of error	The Hälsingborg material						
	Calculated deviation	Number of cases = N	Observed deviation	Number of cases outside the limits of the error of measurement				
			$M \pm \epsilon (M)$	Upwards	% of N	Downwards	% of N	
								$M \pm \epsilon (M)$
Deviation from 11°—21°:								
5—14	3.06 ± 0.30	173	3.29 ± 0.16	109	63.0	2	1.2	
15—24	5.45 ± 0.62	102	6.63 ± 0.53	12	11.8	3	2.9	
25—64	7.70 ± 1.24	184	12.13 ± 0.89	32	17.4	4	2.2	
Deviation from 15°—21°:								
5—14	2.24 ± 0.19	41	2.61 ± 0.26	17	41.5	—	—	
15—24	4.06 ± 0.35	22	4.41 ± 0.65	1	4.5	—	—	
25—64	6.34 ± 0.72	60	7.88 ± 1.42	6	10.0	2	3.3	

10 minutes between the two series. Thereupon they were put aside for sedimenting partly in the laboratory mentioned and partly in an adjoining room with a temperature of about 11° C. (After the 16th of April, 1939, the temperature in this room was about 15° C.)

Some inaccuracies could of course attach to these tests, but they would not be greater than those usually expected to occur at ordinary SR tests. Since two tests were carried out and compared, the results can be directly applied to regular hospital practice.

On this Hälsingborg material were applied the figures previously obtained but computed into increase per degree for the temperatures in the Hälsingborg material. If we compare the calculated increase with the one observed in the Hälsingborg material (Table 5) we find good agreement. This table also gives the differences falling outside the limits of the error of method. For the SR-group of 25—64 allowance has been made for an error latitude of 18 mm upwards and downwards. This latitude, 18.06 mm, was obtained in the following way. The error of method for high SR is 4.26 and the error for a difference between two SR is therefore 6.02. The largest random deviation is thrice this rate, that is to say

Table 6.

Sedimentation rate in 50-year-old woman, suffering from anemia (incompensated vitium cordis).

Date	Sedimentation rate in mm 1 hour at		Hemo- globin in per cent	Red blood corpuscles mill./mm ³	White blood corpuscles thousand /mm ³
	21°	11°			
1938					
28. 12	25	—	86	4.03	7.8
31. 12	25	—	87	3.88	—
1939					
3. 1	57	—	87	3.97	—
7. 1	44	—	86	4.05	—
8. 1	46	—	—	—	—
10. 1	—	—	83	3.92	—
13. 1	47	—	81	3.91	—
16. 1	54	—	85	4.05	—
19. 1	54	—	87	4.00	—
23. 1	63	—	89	4.06	—
26. 1	55	—	90	4.10	—
31. 1	64	51	—	—	—
57. 2	73	83	—	—	—
10. 2	—	—	67	2.76	11.2
14. 2	106	105	—	—	—
17. 2	—	—	67	3.14	6.4
21. 2	73	96	—	—	—
28. 2	101	105	68	3.24	—
7. 3	93	117	—	—	—
9. 3	—	—	66	2.73	—
15. 3	Exitus.				

18.06. This latitude of error is safe because in the Hälsingborg material one and the same test was made use of for deciding the difference between two tests. This produces a smaller standard error than when one test was taken from the right and one from the left arm. We are thus at a safe distance from the margin of error.

We find that at a change in temperature from 11—21° in the group 5—14 mm there is an upward displacement for 63 per cent of the sedimentation tests. In the group 15—24 mm at the same temperature 12 per cent show such a deviation, and in the group

Table 7.

Sedimentation rate in 45-year-old woman, suffering from polyarthritis and in 14-year-old girl, suffering from pleurisy sicca. The tests were made and read off at the same time (\times = Diffuse layer of plasma and blood corpuscles).

Date 1939	Diagnosis			
	Polyarthr. chron.		Pleurisy sicca	
	Temperature			
	21°	11°	21°	11°
21. 2	70	4	—	—
22. 2	62	3	28	18
2. 3	29	9	28	18
15. 3	60	5	—	—
16. 3	30	1	—	—
17. 3	61	3	—	—
18. 3	34	1	—	—
21. 3	38	2	—	—
23. 3	42	3	22	11
29. 3	50	22	—	—
6. 4	43	3	—	—
13. 4	70	14		
Temperature				
	21°	15°	21°	15°
20. 4	51 ×	74	25	20
21. 4	61	14	—	—
27. 4	69	5	21	17
3. 5	61	91	—	—
Temperature				
	22°	18°	22°	18°
11. 5	70	87	30	17
16. 5	63	80	23	17
25. 5	57	85	22	15
30. 5	70	80	—	—

25—64 mm 17 per cent. From 15—21° an increase is found for 40 per cent of the individuals in the group 5—14 mm, 5 per cent of the individuals in the group 15—24 mm and 10 per cent of the individuals in the group 25—64 mm. A downward displacement, i.e. SR against the rule is obtained from 11—21° in 1 per cent of

Table 8.

Sedimentation rates of tests made up of plasma and blood corpuscles from 4 individuals. Tests were made on all the individuals and also with plasma from one individual and blood corpuscles from another. P = plasma. (The Indexfigures show from which individual the plasma was taken.) B = blood corpuscles. (The Indexfigures show from which individual the blood corpuscles were taken.)

Test		Sedimentation rate in mm after			
		$\frac{1}{2}$ hour		1 hour	
		temperature		temperature	
		12°	25°	12°	25°
P = 0.6, B = 0.2 cm ³ in the mixed tests:					
SR according to the rule	1 (unmixed)	2	4	6	15
	P ₁ + B ₁	14	25	32	62
	P ₁ + B ₂	70	83	82	93
SR against the rule	2 (unmixed)	84	51 ¹	108	110
	P ₂ + B ₂	85	70 ¹	102	103
	P ₂ + B ₁	76	35	91	79
P = 0.8, B = 0.3 cm ³ in the mixed tests:					
SR according to the rule	3 (unmixed)	2	2	3	5
	P ₃ + B ₃	6	12	15	27
	P ₃ + B ₁	21	85	55	107
SR agai st the rule	4 (unmixed)	90	90	111	98
	P ₄ + B ₁	85	70	110	123
	P ₄ + B ₃	55	45	109	87

¹ The upper limit of the column of blood corpuscles was diffuse.

the tests for the group 5—14 mm and in 2—3 per cent for the remaining groups. For 15—21° this downwards displacement in the cases under review is only discernible in the SR-group 25—64 mm and with a frequency of about 3 per cent.

Some specially noteworthy cases out of the Hälsingborg material.

1. J. No. 1807—38. A woman aged 50 years with uncompensated vitium cordis which resulted in death. In Table 6 SR and the figures for hemoglobin as well as the number of red and white blood corpuscles have been given. Here we find that during the time when the

patient was suffering from pronounced anemia there was clear evidence of «positive kalte SR». The rates for 21st February and 7th March 1939 lie outside the limits of the error of the method.

This observation agrees with information furnished by Carlinfanti and Balestieri (1938) as well as by Sato and Otuka (1941).

2. J. No. 378/39. A woman aged 45 years with primary chronic polyarthritis. (Table 7). By way of comparison a case of pleuritis sicca is shown where the tests were made and read at the same time. We notice extreme differences here although to begin with they are according to the rule. Later on, however, the SR course changes and we get SR against the rule. This even applies to a temperature difference of only 4° C.

Some experiments with mixed blood corpuscles and plasma.

A «mixed» series will be found in Table 8. As regards the technique, see among others Gripwall (1938). As will be seen from the table, SR against the rule are obtained in those tests where the plasma emanates from a patient with this form of reaction. These experiments would seem to indicate that the properties of SR against the rule or at least the greater part of them, are due to the plasma. To deal at any greater length with the theoretical question of this phenomena lies outside the scope of the present paper.

It may be mentioned here that Sato and Otuka (1941) have suggested a method with concentrated citrate solution whereby SR-test becomes less susceptible to changes in temperature.

Summary.

The standard temperature for sedimentation reactions is usually stated to be a room temperature of about 18° C. (Westergren and others). A higher temperature usually gives a higher SR. Cases will, however, occur when the position is reversed whereby a lower SR is found at a higher temperature. Some authors have made reference to this condition without, however, making any closer examination of it, nor do we find any calculations with reference to the error of measurement.

1) The error of measurement for Westergren's technique has been examined with reference to room temperatures of 12°, 18° and 25° C. The error in mm for the various temperatures reveals

no significant variation. (Table 1 A).—The standard error, calculated as a percentage, consequently is slightly smaller when the temperature is higher (Table 1 B).

2) At rising temperatures the course of SR shows a mean increase after 1 hour as well as after 2 hours (Table 3). The increase is smaller from 12—18° than from 18—24°. (Table 4.) From 12—18° the increase per degree for the group 5—14 mm is 0.21 ± 0.043 mm and from 18—25° 0.54 ± 0.047 mm. In the group 15—24 mm the corresponding rates are 0.35 ± 0.098 mm and 1.0 ± 0.063 mm, and for the group 25—64 mm 0.35 ± 0.19 mm and 1.77 ± 0.14 mm. It is therefore better to keep the temperature somewhat below 18° C. rather than above this level.

3) An examination of a series of hospital cases reveals a positive upward displacement in 10—60 per cent of the cases from 11—21° and 5—40 per cent from 15—21° (Table 5). A downward displacement (i.e. contrary to the rule) occurs in 1—3 per cent from 11—21° whilst for 15—21° this displacement is only discernible for high SR (25—64 mm).

4) Table 7 shows a case with extreme differences of SR according to the rule.

5) Some experiments (Table 8) indicate that the properties of SR contrary to the rule are due to the plasma.

Appendix.

Apparatus with photoelectric-cell for automatic registration of SR.

The apparatus which have hitherto been constructed for automatic registration of SR are all built on the principle of photography.

Many such apparatus have been constructed for various SR methods. We have for instance the »Sedigraph» according to Stammreich (see Gollnow, 1932), »The Sedimentometer» according to Lee (1938). There are further constructions among others by Frimberger (1937), Nichols and Colombus (1938), Sechi (1933) and by Sullkowitch (1934).

For the Westergren technique which was beginning to be adopted abroad on an increasing scale there are only a few apparatus

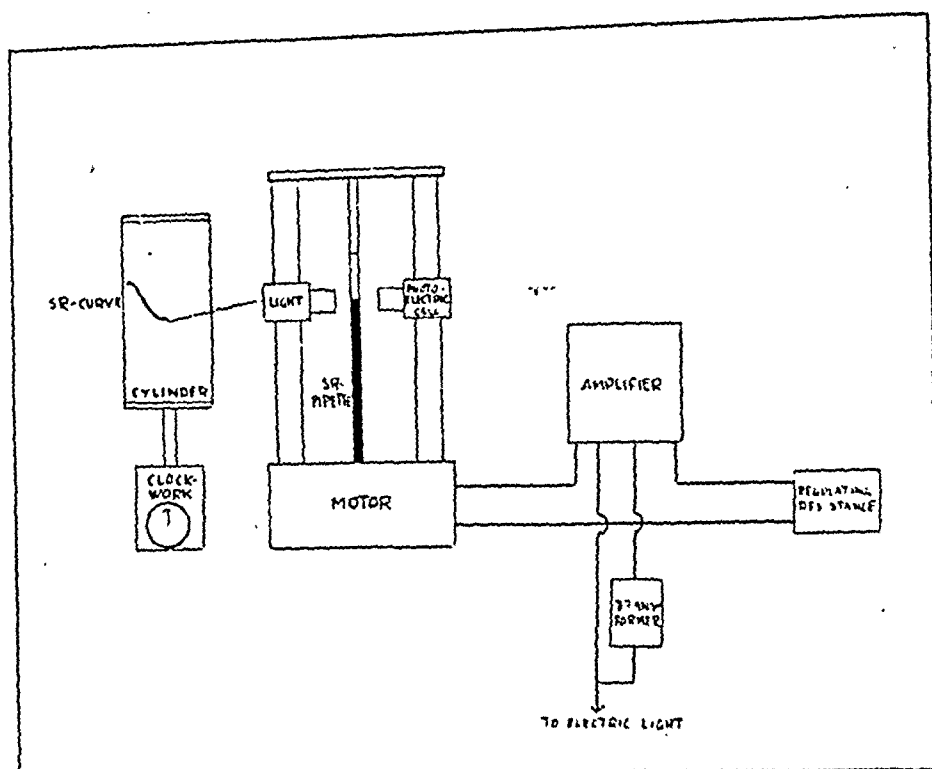


Fig. 1. Schematic drawing of photo-electric apparatus for automatic registration of one sedimentation test.
(According to Visomat «Niveau-Abtaster»).

in existence, notably the «Sediphot» according to Sachs (1932) and an apparatus according to the Swede Lundgren (1927).

«Sediphot» can register 3 tests simultaneously but not longer than 2 hours. One of the advantages of this apparatus is that a light-sensitive paper is employed which requires no developing.

Those apparatus which register only one test at a time reproduce in turn the entire course of SR, and usually across a fairly long period.

With all the apparatus mentioned here the test has to be placed into a dark chamber. As, according to Lenzi (1934), SR is hampered by darkness, the chances are that somewhat erroneous rates are thereby obtained which would possibly not always bear unreserved comparison with tests performed in the ordinary way.

The apparatus of which a rough sketch (fig. 1) is shown here is built on the principle of registration by means of the photo-electric-cell. (Swedin, 1938, has constructed a photo-electrical appa-

ratus for measuring the absorption of light in a vessel containing blood, whereby an analysis of the course of aggregation can be obtained.) The registration can be made in a light room. After the test has been placed on a Westergren stand as usual, a clock is set for the time the reading of the test is wanted. At the appointed time the photo-cell-aggregate is brought into position by a relay and a motor, whereupon the reading will be done automatically with an apparatus on the principle of »Niveau-Abtaster». As the photo-cell reacts at the grading marked »Air/Plasma» as well as at »Plasma/Blood Corpuscle Mixture» an accurate reading is obtained regardless whether the test was drawn exactly to the mark at 200 mm. The apparatus is intended for 20 tests.

For ordinary laboratory purposes, where only one and two hours' rates are required, it is proposed to have the registering done on a calculating machine giving at once the figure for the value of SR, which might also be stamped on a sheet on paper (the journal) by a simple manipulation.

For scientific needs where it may be necessary to follow the course of SR, the construction is designed for about ten tests which are registered every five minutes on a sheet of millimetre-paper attached to a timed drum, by means of a needle on the photocell aggregate.

The reading is done to within 0.1 mm.

For other methods for Sedimentation Reaction it should be possible to work out practical apparatus on the photo-cell principle. These should prove simpler and cheaper than for Westergren's technique. An apparatus for Linzenmeier's (1920) technique would probably be the simplest, the range for sedimentation being constant and time the changing factor.

(I should like to extend my best thanks to Mr. E. Dingel, Director of »Kifa» and Engineer C. Ahlberg of Messrs. L. M. Ericsson, who carried out the experiments with photoelectric-cell, and to Mr. Holmsen and Mr. H. Palm, engineers at »Kifa», for working out the constructional details.)

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Aus der III. Medizinischen Klinik der Universität Helsinki.
Vorstand: Prof. W. Kerppola.

Über durch Gonorrhoe bedingte Gelenkentzündungen.

Von

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(Bei der Redaktion am 25. Februar 1944 eingegangen).

Die Diagnose und Therapie der durch Gonorrhoe bedingten Gelenkentzündungen sind nach dem Jahre 1930 dadurch hochgradig gefördert worden, dass man in der Gonokokken-Komplement-bindungsreaktion (Gonoreaktion) ein praktisches Untersuchungsmittel bei derartigen Gelenkerkrankungen und in den Sulfonamidpräparaten ein vorzügliches Behandlungsmittel gefunden hat. Die Effektivität der Chemotherapie und der Umstand, dass die leichten und polyartikulären Krankheitsformen besser als früher diagnostiziert werden können, bewirken, dass die Behandlung der Krankheit von den Chirurgen immer mehr an die Internisten übergeht.

Die in der III. Medizinischen Klinik der Universität Helsinki gemachte Erfahrung, dass die gonorrhoeischen Gelenkentzündungen im Vergleich zu den rheumatischen viel häufiger sind, als man auf Grund der über die Häufigkeit der Krankheit verbreiteten Auffassung erwarten sollte, veranlasste die folgende Untersuchung. Da sich das zur Verfügung stehende Krankengut als relativ gross erwies und sich bei der Untersuchung interessante Umstände über die Häufigkeit, die Pathogenese und die klinischen Symptome der durch Gonorrhoe bedingten Gelenkentzündungen herausstellten, erscheint die Publikation gerechtfertigt.

Auswahl und Zusammensetzung des Krankengutes.

Das Krankengut stammt aus dem Kivelä-Krankenhaus und der dort untergebrachten III. Medizinischen Klinik der Universität Helsinki. Ich bitte dem Chefarzt der medizinischen Abteilung des Kivelä-Krankenhauses, Herrn Doz. P. Soisalo, meinen Dank dafür aussprechen zu dürfen, dass er mir das Patientenmaterial der medizinischen Abteilungen des Krankenhauses, die nicht zur III. Medizinischen Universitätsklinik gehören, überlassen hat. In das Krankengut sind alle im Krankenhaus in den Jahren 1936—1942 wegen Arthritis behandelten Kranken aufgenommen, bei denen während der Gelenkerkrankung oder kurz vor deren Beginn Gonorrhoe durch Bakterienuntersuchung nachgewiesen wurde oder bei denen die Gonoreaktion (GR) nach der Methode Kristensens während der Gelenkentzündung stark positiv gewesen ist ($GR > 3$). End der Gelenkentzündung beträgt 136. Bei 74 von diesen war die Zahl der Erkrankten beträgt 136. Bei 74 von diesen war die Gonorrhoe bakteriologisch festgestellt worden; die übrigen 62 wurden lediglich auf Grund einer stark positiven GR in die Untersuchung einbezogen. Unter den letzteren hatte die Gonokokkenuntersuchung, die sich meistens auf die Untersuchung einer Probe gründete, bei 32 ein negatives Ergebnis; bei den anderen war sie nicht ausgeführt worden. Es ist besonders angebracht, zu erwähnen, dass in der Anamnese oder im Status von 30 der 62 Patienten, die lediglich auf Grund einer stark positiven GR in die Untersuchung einbezogen wurden, Umstände vorkommen, die ausdrücklich für die Möglichkeit einer Gonorrhoe sprechen, wie Fluor albus, Miktionsbeschwerden und Eierstocksentzündung. Die Gonoreaktionsuntersuchungen wurden im Sero-Bakteriologischen Institut der Universität ausgeführt.

Am einfachsten wäre es natürlich, wenn das Krankengut alle in den Jahren 1936—1942 im Krankenhaus behandelten gonorrhoischen Gelenkentzündungsfälle umfasste. Die Zahl derselben lässt sich jedoch nachträglich nicht genau ermitteln, weil die ausgeführten Untersuchungen — teilweise infolge der durch den Krieg bedingten Verhältnisse — in vielen Fällen unvollständig geblieben sind, trotzdem man der Klarlegung der Ätiologie der Gelenkentzündungen in dem erwähnten Krankenhaus schon vom Jahre 1937 an besondere Aufmerksamkeit gewidmet hat, was u.a. aus der Zahl der GR-Untersuchungen hervorgeht (S. 5). Die

gonorrhöische Gelenkentzündung hat bisweilen unerkannt bleiben können; andererseits wären in vielen Fällen, wo die Krankenhausdiagnose »gonorrhöische Arthritis« lautete, weitere Untersuchungen zur Sicherstellung der Diagnose nötig gewesen.

Kristensen ist der Ansicht, dass eine Gonoreaktion vom Stärkegrad 3 oder darüber nur in Ausnahmefällen unspezifisch ist. Spätere Untersuchungen haben gezeigt, dass die Behauptung Kristensens in grossen Zügen stichhaltig ist. Bang und Krag, die bei 1895 Patienten eines dänischen Krankenhauses für innere Krankheiten in 3.2 % »starke« Reaktionen (GR 3 und darüber) antrafen, konstatierten bei 90 % den letzterwähnten eine Gonorrhoe oder Umstände, die für die Möglichkeit einer Gonorrhoe sprachen. Der Hundertsatz der Wahrscheinlichkeit betrug 63 bei Personen mit GR 1, 71 bei solchen mit GR 2, 85, wenn GR 3 und weit über 90, wenn ein höherer GR-Wert vorlag.

Die Sicherstellung der Diagnose durch Gonokokkenbefund ist bei gonorrhöischen Gelenkentzündungen oft schwierig oder unmöglich, obgleich die positive Gonoreaktion durch Gonorrhoe bedingt ist. So ist der Sachverhalt besonders in solchen Fällen, wo die Gonorrhoebehandlung eingeleitet war, ehe der Kranke wegen seiner Gelenkentzündung ins Krankenhaus gelangte. Eine Gonoreaktion, deren Stärkergrad 3 übersteigt, ist mit grosser Wahrscheinlichkeit durch eine Gonorrhoe verursacht. Die fortgesetzte Verfolgung des Stärkegrades der Gonoreaktion bringt auch darüber Aufschluss, ob die Reaktion durch Gonorrhoe bedingt ist oder nicht. Die unspezifischen Reaktionen sind im allgemeinen von kurzer Dauer. Man kann auch nicht immer mit Bestimmtheit entscheiden, ob die Erkrankung eines Patienten an Gonorrhoe einen Anteil an der Entstehung der Gelenkentzündung hat, weil der Kranke gleichzeitig an Gonorrhoe und an einer, nicht durch Gonorrhoe bedingten Gelenkentzündung leiden kann. Der Umstand, dass bei Patienten, die an einer akuten Arthritis leiden, die positive GR und die Gonorrhoe viel häufiger sind als bei anderen Patienten, lässt sich jedoch nicht anders erklären, als dass die Gelenkentzündung in diesen Fällen meist in ursächlichem Zusammenhang mit der Gonorrhoe steht. Die Zahl der Fälle, in denen es sich um ein blosses Zusammentreffen zweier verschiedener Krankheiten handelt, kann sich nur auf einen Bruchteil der Gesamtanzahl belaufen.

Aus den obenerwähnten Gründen sind die Patienten lediglich auf Grund bestimmter objektiver Symptome — eines positiven Gonokokkenbefundes und einer stark positiven Gonoreaktion — in das Krankengut aufgenommen worden. Beim grössten Teil der Fälle des so zusammengebrachten Materiales hat es sich offenbar um eine gonorrhöische Gelenkentzündung gehandelt. Wegen der Unspezifität der Gonoreaktion oder infolge des Zusammentreffens einer Gonorrhoe und einer, auf irgendeiner andern Ätio-

logie beruhenden Gelenkentzündung können jedoch auch einige Fälle dabei sein, die in Wirklichkeit nicht von einer Gonorrhoe herrührten.

In mein Krankengut gehen insgesamt 96 Fälle oder 70 % Frauen und 40 Fälle oder 30 % Männer ein. Die grosse Anzahl der Frauen beruht in erster Linie darauf, dass das Patientenmaterial des Kivelä-Krankenhauses auch im allgemeinen überwiegend aus Frauen besteht. In den Jahren 1936—42 haben die Frauen 61 % und die Männer 39 % der Gesamtanzahl der behandelten Kranken ausgemacht. Aus dem mir zur Verfügung stehenden Krankengut glaube ich schliessen zu dürfen, dass die gonorrhoeische Arthritis

Tabelle 1.

Alter	Anzahl der Patienten
—20 J.	16
21—30 J.	62
31—40 J.	40
41—50 J.	14
51—	4

bei Frauen nicht seltener als bei Männern auftritt. Mondor, Kowarschik und Laitinen sind zu ähnlichen Ergebnissen gekommen. Die frühere Auffassung, dass die Krankheit bei Frauen seltener als bei Männern ist, rührt wohl davon, her, dass die Gonorrhoe vor Einführung der GR bei Frauen relativ öfter als bei Männern undiagnostiziert geblieben ist.

Wie aus Tab. I erhellt, ist der grösste Teil der Patienten unter 40 Jahre alt gewesen:

Die Häufigkeit der gonorrhoeischen Gelenkentzündungen.

Die Zahl der wegen akuter Gelenkentzündungen in den Jahren 1936—42 im Kivelä-Krankenhaus behandelten Patienten beläuft sich auf 448, was ca 2 % der Gesamtanzahl der in der besagten Zeit behandelten Patienten ausmacht. Die Zahl der wegen chronischer Gelenkkrankheiten in derselben Zeit behandelten Kranken ist doppelt so gross.

In 130 Fällen des Krankengutes ist die Gelenkentzündung akut gewesen, so dass ca 30 % der Patienten des Krankenhauses, die eine akute Gelenkentzündung gehabt haben, in das Krankengut eingehen. Zu beachten ist ferner, dass einige undiagnostiziert gebliebene gonorrhoische Gelenkentzündungsfälle dieser Untersuchung entgangen sein können. Es ist offenbar, dass ein erheblicher Teil der suspekten Gonoreaktionen (GR 1—3) auch bei den Kranken, bei denen keine Gonokokken gefunden oder gesucht wurden, in Wirklichkeit durch eine Gonorrhoe verursacht war. 10 solcher Patienten mit GR 3 sind während der Jahre 1936—42 im Krankenhaus gelegen. Die Zahl der in das Krankengut aufgenommenen, ebenso wie der im Krankenhaus als gonorrhoische Arthritis diagnostizierten Fälle war in den Jahren 1937—39, wo die GR bei 4/5 aller an akuter Gelenkentzündung leidenden Kranken angestellt wurde, bedeutend grösser als i.J. 1936 und in den Jahren 1940—1942, wo die GR nur bei der Hälfte der diesbezüglichen Kranken ausgeführt wurde. Aus den ersterwähnten Jahren gelangten ca $\frac{1}{3}$, aus den letzterwähnten ca $\frac{1}{4}$ der Patienten mit akuter Gelenkentzündung in das Krankengut. Die Zahl der hier aufgenommenen Fälle hing demnach davon ab, wie sorgfältig die Patienten untersucht worden sind.

Aus dem oben Ausgeführten geht hervor, dass ein Drittel bis ein Viertel der im Krankenhaus behandelten akuten Gelenkentzündungen durch Gonorrhoe bedingt war.

Auf Grund der von den Ärzten gemachten Anzeigen gab es in Finnland i.J. 1938 8450 frische Gonorrhoeefälle, hiervon 3286 in Helsinki (Olin). Die wirkliche Zahl der Fälle ist jedoch offenbar bedeutend grösser gewesen. Weil die Gonorrhoe, ebenso wie die Lues, in der Hauptstadt viel häufiger als anderswo im Lande sind, muss man in Helsinki auch mehr gonorrhoische Gelenkentzündungen als in der Provinz erwarten.

In Dänemark haben Bang und Krag die Häufigkeit der positiven Gonoreaktion bei Gesunden und Kranken untersucht. Unter 534 »gesunden« Kopenhagener Blutspendern fanden sie in 2.7 %, unter 1895 inneren Patienten in 6.7 % eine positive GR. Von 54 Patienten mit akuter Gelenkentzündung hatten 13 eine positive GR. Man muss hierbei jedoch berücksichtigen, dass die Hälfte der Fälle aus solchen bestand, bei denen die Gradstärke der Reaktion 1—3 betrug, die Spezifität der Reaktion also suspekt war.

Über die auf die Entstehung der gonorrhoeischen Gelenkentzündungen einwirkenden Faktoren.

28 von den 136 Kranken des Krankengutes haben schon früher Gelenkentzündungen gehabt. In 23 Fällen ist die frühere Arthritis durch eine Gonorrhoe bedingt gewesen oder ist wenigstens die Möglichkeit einer solchen vorgelegen. In 5 Fällen hat die frühere Gelenkentzündung schon im Kindesalter bestanden, ist also wahrscheinlich rheumatischer Natur gewesen, so dass man bei diesen Patienten eine Neigung zu verschiedenartigen Gelenkkrankheiten annehmen muss.

Ein Trauma ist der Gelenkentzündung in keinem Fall unmittelbar vorausgegangen. In 2 Fällen hat sich die Entzündung indessen vorzugsweise in solchen Gelenken lokalisiert, die schon Jahre vorher von einem Trauma betroffen oder durch Krankheit geschädigt waren.

Aus der Untersuchung geht hervor, dass der Beginn der Krankheit ungefähr gleich oft auf die verschiedenen Monate entfallen ist. Dies stimmt gut mit der Feststellung überein, dass die Gonorrhoe in Helsinki ungefähr gleichmässig über das ganze Jahr verteilt ist.

In 18 Fällen hat der Patient einige Tage oder Wochen (3—30 Tage) vor Beginn der Gelenkerkrankung Halsschmerzen gehabt. Obgleich es sich in einigen Fällen um eine Gonorrhoe und eine gleichzeitige rheumatische Gelenkentzündung mit einer ihr vorausgehenden Angina handeln könnte, muss man in den meisten Fällen die Diagnose gonorrhoeische Arthritis auf Grund der Anamnese, des »klassischen« klinischen Bildes, des Gonokokkenbefundes und der stark positiven GR doch für erwiesen halten. Es scheint also, dass eine unspezifische Infektion zur Entstehung von Gelenkentzündungen bei Gonorrhoe-Patienten beitragen kann.

Das klinische Bild.

Die Gelenkentzündung ist in 130 Fällen akut und in 6 Fällen chronisch oder subchronisch verlaufen.

Im allgemeinen gehörte Fieber zum klinischen Bilde. Bei einem Viertel der Kranken ist jedoch die Temperatur im Kran-

kenhaus nicht über 37.2° (axil.) gestiegen; aber die meisten von ihnen hatten vor ihrer Aufnahme ins Krankenhaus Fieber gehabt, Der Wert der Senkungsreaktion war in allen akuten Fällen erhöht, in den meisten stark erhöht.

Die Gonoreaktion wurde in 127 Fällen ausgeführt. Stark positiv (> 3) war sie in 112, »suspekt« (1—3) in 7 und negativ in 8 Fällen. Wie früher bereits erwähnt worden ist, war der positive Ausfall der Gonokokkenuntersuchung die Voraussetzung für die Aufnahme solcher Fälle ins Krankengut, bei denen die GR suspekt oder sogar negativ war. Obgleich die GR in den meisten derartigen Fällen in einem zu frühen Stadium der Gelenkkrankheit angestellt war, ist sie in 3 Fällen noch über 2 Wochen nach dem Beginn der Gelenkentzündung negativ gewesen. Man könnte natürlich vermuten, dass es sich in solchen Gelenkentzündungsfällen, wo die GR negativ bleibt, trotzdem bei dem Kranken eine Gonorrhoe festgestellt ist, um das Zusammentreffen einer Gonorrhoe mit einer Gelenkentzündung aus anderer Ätiologie gehandelt hätte. Von den 150 Fällen Walters waren 4 dieser Art. Dafür, dass die Gelenkentzündung auch in solchen Fällen gonorrhöisch sein kann, spricht ein Fall, bei dem die GR während der ganzen Krankheit nicht stärker als 3 wurde, obwohl in der Gelenkflüssigkeit Gonokokken nachgewiesen wurden.

Aus Tab. 2 ersieht man den Stärkegrad der GR in den Fällen des Krankengutes, wo die Reaktion angestellt wurde:

Tabelle 2.

Stärkegrad der GR	0	1	2	3	4	5	6	7	8	9	10	11	12	13	—13
Anzahl der Patienten	8	2	1	4	14	10	21	19	14	8	6	9	7	4	127

Was die *Gelenksymptome* anbelangt, so ist bei der Untersuchung ein Unterschied zwischen den subjektiven (Schmerzen und Empfindlichkeit) und den objektiven Gelenksymptomen (Gelenkschwellungen) gemacht worden. Bei 60 % der Patienten bestanden, als sie ins Krankenhaus kamen, objektive Gelenksymptome an einem Gelenk oder überhaupt keine objektive Gelenksymptome, bei 20 % an zwei und bei 20 % an 3 oder mehr Gelenken. Ausserdem sind bei manchen Patienten vor der Aufnahme ins Krankenhaus oder

später im Krankenhaus objektive Symptome an Gelenken aufgetreten, die bei der Ankunft der Patienten ins Krankenhaus symptomfrei waren. Bei insgesamt 30 % der Kranken sind während der Krankheit objektive und bei über 50 % subjektive Symptome an 3 oder mehr Gelenken vorgekommen. Ich möchte besonders den Umstand unterstreichen, dass die Ergebnisse der Gonokokkenuntersuchung bei der polyartikulären Krankheitsform relativ ebenso oft positiv gewesen sind wie bei der mono- und der biartikulären.

Die Gelenkentzündung ist meistens in den Knien, den Fuss- oder den Handgelenken lokalisiert. Sehr gewöhnlich ist die Gelenkentzündung auch in einem Schulter-, Ellbogen-, Finger- oder Zehengelenk gewesen. Nur in einzelnen Fälle sind Symptome seitens der Sternoklavikular-, Hüft-, Sakroiliakal- oder der Wirbelgelenke vorgelegen.

Die allgemeine Auffassung war und ist, dass bei den gonorrhoischen Gelenkentzündungen im Anfang der Krankheit kurzdauernde, von einer Stelle zur andern wandernde Schmerzen und Empfindlichkeiten in mehreren Gelenken bestehen, dass sich aber die Entzündung im späteren Stadium der Krankheit nur in einem oder wenigen Gelenken lokalisiert. Viele Forscher (Thomas, Wehrbein, Kingreen, Kowarschik, Keefer & Wesley und Wanderer) haben jedoch die Häufigkeit der polyartikulären Krankheitsformen betont. Aus den Publikationen geht indessen nicht hervor, wie gross die Zahl der Patienten war, bei denen objektive Symptome an mehreren Gelenken festgestellt wurden.

Aus der von mir ausgeführten Untersuchung erhellt, dass auch solche polyartikuläre Krankheitsformen, bei denen objektive Symptome an 3 oder mehr Gelenken festgestellt werden, verhältnismässig häufig sind. Es ist sehr verständlich, dass die polyartikulären Krankheitsformen in den chirurgischen Krankenhäusern seltener als in den Krankenhäusern für innere Krankheiten anzutreffen sind. Im allgemeinen bleiben sie auch leichter als die monoartikulären Formen undiagnostiziert, weil es ohne Spezialuntersuchung unmöglich ist, sie von den rheumatischen Gelenkentzündungen zu unterscheiden.

Symptome einer *Tendovaginitis*, *Bursitis* oder *Periostitis* wiesen 17 Patienten neben den Gelenksymptomen auf. In einigen Fällen wurde das Krankheitsbild von derartigen peri- oder extra-

artikulären Symptomen beherrscht, von denen die Fersenschmerzen am gewöhnlichsten sind.

Ischiassymptome (ausstrahlende Schmerzen in den unteren Extremitäten, Druckempfindlichkeit im Bereich des N. ischiadicus, positiver Lasègue) werden bei 5 Kranken entweder gleichzeitig mit Gelenksymptomen oder nach denselben angetroffen. Vom Standpunkt der Praxis ist es wichtig zu wissen, dass die durch Gonorrhoe bedingten Neuralgien und Neuritiden keine grossen Seltenheiten sind.

Herzkomplikationen werden bei den Patienten des Materials sehr selten angetroffen.

Die 3 Patienten, bei denen ein Klappenfehler festgestellt wurde, hatten schon früher eine Gelenkentzündung gehabt, in zwei Fällen bereits als Kind. Wenigstens in den letzterwähnten Fällen ist der Klappenfehler wahrscheinlich rheumatischen Ursprungs. Bei elektrokardiographischen Untersuchungen, die leider nur bei einem Teil der Kranken ausgeführt worden sind, findet man auf eine Myokardschädigung hinweisende Veränderungen selten. In einigen Fällen ist jedoch die T-Zacke in der I. oder II. Ableitung während der Krankheit niedrig oder isoelektrisch. Bei einem Patienten wurde ein elektrokardiographischer Befund angetroffen, der an Vorderwandinfarkt erinnert. Atrioventrikuläre Leitungsstörungen, die bei rheumatischen Myokardentzündungen sehr gewöhnlich sind, liegen nur in einem Fall vor. Auch dieser Kranke hatte 3 Wochen vor seiner Gelenkerkrankung eine Angina gehabt, und war danach dauernd müde und fiebernd gewesen.

Bei den rheumatischen Gelenkkrankheiten gehören die Herzkomplikationen direkt zum Krankheitsbild. Von den Patienten des Krankengutes hatten, wie erwähnt, 28 schon früher Gelenkentzündungen gehabt. Sofern es sich hierbei im allgemeinen um eine rheumatische Gelenkentzündung gehandelt hätte, wäre zu erwarten gewesen, dass die Patienten mehr Klappenfehler und Herzmuskelerkrankungen gehabt hätten, als es der Fall war. Obgleich die im Krankenhaus ausgeführten Herzuntersuchungen teilweise nicht nach unseren Gesichtspunkten durchgeführt worden sind, ist es offenbar, dass die Endo- und Myokarditiden auch bei gonorrhoeischen polyartikulären Gelenkentzündungen viel seltener, leichter sind und weniger zu Folgeerscheinungen neigen als bei den rheumatischen Gelenkentzündungen.

Bei einem Patienten entwickelte sich während der gonorrhoeischen Arthritis ein typisches *Lungenasthma*:

35-jährige Arbeiterehefrau (Nr 481/41). Im Januar 1941 setzten bei der Patientin Kreuz-, Knie- und Knöchelschmerzen ein. Einige Tage später griffen die Schmerzen auch auf die Hand- und Schultergelenke über, die anschwellen. Ins Krankenhaus kam die Patientin 1 ½ Wochen nach Beginn der Gelenksymptome. Dort wurde folgendes festgestellt: Gc + in der Harnröhre, SR 96 mm, GR 8, Hgb 52 (Sahli), E 3.4 Mill. I 0.76, L. 9300, davon 4 % Eosinophile. Nach dreiwöchigem Krankenhausaufenthalt bekam die Kranke Husten und nächtliche Atemnot, woraus sich allmählich ein typisches Lungenasthma entwickelte. Früher hatte die Patientin niemals solche Symptome gehabt. Bei der Entlassung aus dem Spital, wo sie sich 1 ½ Mon. aufgehalten hatte, waren die Gelenke annähernd symptomfrei und die Patientin frei von asthmatischen Beschwerden. Von den Leukozyten waren damals 16 % Eosinophile. Als Behandlung war u.a. Sulfapyridin angewandt worden. Nach der Entlassung hatte die Patientin noch 2 Mon. lang Asthmasymptome, ist aber 2 Jahre lang symptomfrei gewesen.

Der Verlauf der Gelenkkrankheit ist nur in 6 Fällen chronisch oder subchronisch. Hierbei sind alle Patienten einbegriffen, die über 6 Mon. Symptome einer aktiven Gelenkentzündung darbieten. In den meisten derartigen Fällen ist es schwer zu sagen, ob die Gonorrhoe, an der der Patient litt, einen Anteil an der Entstehung der Gelenkentzündung hatte oder nicht. Dafür, dass die Gonorrhoe chronische Arthritiden verursachen oder wenigstens zu solchen prädisponieren kann, spricht u.a. die Feststellung von Bang und Krag, dass GR, ausser bei den akuten, auch bei den chronischen Gelenkerkrankungen öfter positiv ist als bei den Kranken im allgemeinen.

Behandlung und Behandlungsergebnisse.

Ausser den früher angewandten Behandlungsmethoden sind im Kiveliä Krankenhaus vom Jahre 1938 an Sulfonamidpräparate bei der Behandlung der durch Gonorrhoe bedingten Gelenkentzündungen gebraucht worden. Die Behandlungsergebnisse besserten sich unter deren Einwirkung sogleich erheblich. In den mit Sulfonamidpräparaten behandelten Fällen betrug die Behandlungszeit meistens 2—4 Wochen. Etwa die Hälfte der so behandelten Patienten konnte das Krankenhaus schon nach einem Monat rekonvaleszent verlassen. In den Fällen dagegen, wo keine Sulfonamidpräparate verabreicht wurden, dauerte die Be-

handlungszeit im allgemeinen 1—3 Monate, und nur ein Viertel der diesbezüglichen Kranken verliess das Krankenhaus nach einem Monat. Anfangs wurden von den neuen Arzneien zu niedrige Dosen und auch diese zu selten verabreicht. In dem Masse, als die Dosen vergrössert und die Dosierung auf den ganzen Tag ausgedehnt wurden und man vom Sulfanilamid und Uliron zum Sulfapyridin und Sulfathiazol überging, wurden die Behandlungsergebnisse immer besser. Ausser auf die Behandlungsdauer üben die Sulfonamidpräparate, nach allem zu schliessen, auch auf das klinische Krankheitsbild eine gute Wirkung aus. Die schweren Symptome sind oft rasch verschwunden. In vielen Fällen, wo die Behandlung in einem frühen Stadium begonnen worden ist, hat sich das Krankheitsbild, wahrscheinlich infolge der Behandlung, nicht schwer gestaltet.

Todesfälle sind 5 in unserem Krankengut vorgekommen. Drei der Patienten starben an einer Sepsis, nachdem sie mehrere Wochen gefiebert hatten. Es sei erwähnt, dass GR in allen diesen Fällen stark positiv (11—13) war. Sulfonamidpräparate waren in zwei Fällen gar nicht und in einem Falle nur in ungenügender Menge angewandt worden. Ein über 60 Jahre alter Patient, bei dem GR 13 vorlag, starb unerwartet in der Rekonvaleszenz an eine Lungenembolie. Der fünfte Patient, bei dem die Krankheit tödlich ausging, war ein junger Mann, der an schwerer chronisch deformierender Polyarthrit litt. Der Gonokokkenbefund war im Krankenhaus positiv und die GR 10. Während der Krankheit wurden alle Gelenke deformiert, und der Allgemeinzustand verschlechterte sich dauernd. Der Patient starb 2 ½ Jahre nach Beginn der Krankheit.

Über die endgültigen Behandlungsergebnisse lässt sich in Ermangelung von Nachuntersuchungen nichts Sicheres aussagen, ebensowenig darüber, welche Wirkung die Einführung der Sulfonamidpräparate in dieser Hinsicht gehabt hat. Bei der Entlassung der Patienten aus dem Krankenhaus waren die Gelenke jedoch in den meisten Fällen objektiv symptomfrei. Nur 4 Patienten mit schwerer monoartikulärer Gelenkentzündung mussten in ein chirurgisches Krankenhaus verlegt werden.

Zusammenfassung.

Bei einer Gesamtanzahl von 448 in interne Behandlung genommenen akuten Gelenkentzündungen wurden 130 ($\frac{1}{3}$ — $\frac{1}{4}$ aller im Krankenhaus überhaupt behandelten Fälle), bei denen eine Gonorrhoe oder auf Grund einer stark positiven Gonoreaktion ein Gonorrhoeverdacht bestand, untersucht.

Dass in manchen Fällen auch eine unspezifische Infektion zur Entstehung der gonorrhoeischen Gelenkentzündungen beigetragen haben mochte, musste angenommen werden.

20 % der Patienten boten bei der Aufnahme ins Krankenhaus objektive und über 50 % subjektive Symptome an drei oder mehr Gelenken dar. Tendovaginitiden, Bursitiden oder Periostitiden wurden während der Krankheit bei 17 und Ischiassymptome bei 5 Patienten angetroffen.

Die Diagnose bei Gelenkentzündungen, insbesondere bei polyartikulären Krankheitsformen, ist oft schwierig. Die richtige Diagnose ist besonders wichtig, da die Erfolge der neuesten Chemotherapie (Sulfonamide) eine aussichtsreiche, kausale Therapie ermöglichen einerseits, während andererseits die Prognose der gonorrhoeischen Arthritis im Gegensatz zur rheumatischen wesentlich günstiger gestellt werden kann, da die Herzkomplicationen bei der gonorrhoeischen Arthritis unvergleichlich seltener einzutreten pflegen, und, wenn sie eintreten, doch nur ausnahmsweise zu Spätfolgen neigen.

Es erscheint wichtig die Gonoreaktion bei allen Fällen akuter Gelenkentzündung auszuführen.

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Recherches sur le mécanisme de l'immunité, de l'anaphylaxie et des maladies spécifiques (maladie du sérum, maladies infectieuses).

Phylaxie, paraphylaxie et choc paraphylactique acétylcholinique.

Premier Mémoire.

Critique des hypothèses antérieures, conception personnelle.

Par

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(Ce travail est parvenu à la rédaction le 3 Février 1944.)

Lorsque P. Portier et Ch. Richet ont décrit en 1902 l'anaphylaxie, ces auteurs ont expliqué ce phénomène de la manière suivante.

L'antigène provoque la formation dans l'organisme de *toxogénine* qui, lors de l'injection déchaînant d'antigène, se combine à ce dernier formant ensemble un poison, l'*apotoxine*, qui provoque le choc anaphylactique.

Toxogénie + antigène = apotoxine.

Ch. Richet admet la production dans l'organisme traité par une toxine de deux substances, une substance immunisante, l'*antitoxine* et une substance sensibilisante, la *toxogénine*.

Quant au mécanisme intime du choc anaphylactique l'auteur affirme qu'il ne s'agit pas d'un «*conflit de la substance injectée avec le protoplasme vivant . . . mais tout simplement de l'épuisement de la substance toxogénique*».

Le choc anaphylactique serait «*une intoxication aiguë du système nerveux*».

Se demandant *»comment l'anaphylaxie se concilie avec la loi de défense de l'organisme»* Ch. Richet énonce l'hypothèse suivante.

La défense de l'organisme comporte, non seulement, la défense de l'individu, mais aussi la défense de l'espèce. Il faut que l'être reste stable. *»C'est pour le maintien de cette stabilité, qu'il réagit avec toute l'énergie aux atteintes chimiques qui peuvent l'affecter».*

Selon M. Arthus, M. Nicolle et beaucoup d'autres auteurs l'anaphylaxie et l'immunité sont deux états tout à fait distincts et opposés. M. Nicolle décrit deux catégories d'anticorps: les anticorps coagulants qui produisent l'immunité et les anticorps lytiques qui produisent l'anaphylaxie.

Le choc anaphylactique est expliqué par la production d'un poison, l'anaphylatoxine (Friedberger) qui est libérée par l'alexine à partir de l'antigène sensibilisé par l'anticorps sensibilisant. Bordet admet aussi la production d'une sensibilisation de l'organisme et explique le choc anaphylactique par la production d'anaphylatoxine qui serait tirée (par un phénomène d'adsorption) de l'alexine par le complexe antigène-anticorps. Il affirme qu'une simple suspension de gélose injectée dans la veine est capable (par un phénomène d'adsorption) de libérer à partir de l'alexine le poison appelé anaphylatoxine et de provoquer ainsi le choc anaphylactique.

Von Pirquet explique la maladie du sérum par la production d'anaphylatoxine libérée à partir de l'antigène sensibilisé par l'anticorps. Cet auteur et ensuite Friedberger ont expliqué les phénomènes cliniques des infections par un mécanisme semblable: tous ces phénomènes sont l'effet de l'anaphylatoxine tirée de l'antigène (bactérie) sensibilisé par l'anticorps. La maladie du sérum et les infections sont par conséquent d'après ces auteurs des phénomènes de choc anaphylactique pur.

D'autres hypothèses ont été encore formulées pour expliquer le mécanisme de l'anaphylaxie. Mais consultant les traités de spécialité nous constatons que l'opinion courante, devenue classique, est la suivante.

1° Tout antigène provoque dans l'organisme deux états tout à fait distincts, l'un d'anaphylaxie et l'autre d'immunité.

2° Les phénomènes anaphylactiques sont opposés aux phénomènes d'immunité.

3° L'organisme anaphylactisé est hypersensible vis-à-vis d'un antigène, dans le sens que cet antigène provoque, chez l'animal

préparé par le même antigène, des phénomènes toxiques plus intenses que ceux qu'il provoque chez l'animal neuf.

4° L'antigène provoque la formation de deux catégories d'anticorps, les uns immunisants, les autres anaphylactisants.

5° Le choc anaphylactique est dû à la production d'une anaphylatoxine.

6° Tous les phénomènes qui prennent naissance, pendant le choc anaphylactique, sont dus à la production d'histamine.

Tout est erroné dans cette conception. Nous avons vécu quarante ans sur une grande erreur qui a conduit les auteurs à des conclusions tout à fait illogiques et qui ont empêché tout progrès sérieux dans l'étude du problème.

A. Critique des hypothèses antérieures.

1° On ne peut pas admettre que l'organisme devienne plus sensible vis-à-vis de l'antigène, pendant qu'il se défend contre cet antigène. Etudiant les réactions des tissus contre les différentes substances, antigéniques ou non antigéniques, introduites dans l'organisme, nous sommes arrivés à la conviction *qu'il ne se produit jamais que des réactions de défense* et qu'on ne peut pas admettre l'existence d'une sensibilisation.

Richet croit que l'organisme est en même temps immunisé et sensibilisé, car l'auteur définit l'anaphylaxie «la curieuse propriété que possèdent certains poisons d'augmenter, au lieu de diminuer, la sensibilité de l'organisme à leur action».

Bordet ne croit pas que l'anaphylaxie est le contraire de l'immunité. Il admet pourtant l'existence de l'anaphylaxie ainsi que la production d'un poison, l'anaphylatoxine. Mais tous les auteurs croient à un état d'hypersensibilité, état opposé à l'immunité, coexistence qui à notre avis est inadmissible.

Nous verrons d'ailleurs plus loin qu'aucune des expériences antérieures ne prouve l'existence de l'anaphylaxie, telle qu'on l'a conçue jusqu'à présent et nos recherches sur l'action des substances étrangères introduites dans l'organisme nous permettent d'affirmer que ce dernier ne réagit que par des phénomènes d'immunité.

2° Selon la conception classique l'antigène provoque un état d'immunité spécifique et un état d'anaphylaxie spécifique. Si

cette conception était vraie, et se l'anaphylaxie était spécifique, il faudrait que la symptomatologie du choc anaphylactique soit différente suivant l'antigène qui est en cause. Or, elle est identique ou du moins comprend des symptômes identiques. Comment pouvons-nous concevoir qu'un choc anaphylactique spécifique soit symptomatiquement identique avec une albumine étrangère, ou avec des hématies ou avec des bactéries?

Nous avons prouvé, d'un autre côté, que l'état d'anaphylaxie que provoque un antigène injecté à un animal est un état végétatif anormal, car le tonus ainsi que la réactivité des organes à différents facteurs végétatifs sont modifiés (vis-à-vis de l'état normal). Si l'anaphylaxie était un état spécifique, dû à des anticorps spécifiques, chaque antigène devrait provoquer un état anaphylactique spécifique, différent selon l'antigène qui a été introduit dans l'organisme. Or, l'état anormal provoqué par tous les antigènes aussi différents qu'ils soient les uns des autres, est identique. Nous verrons qu'en réalité, il n'y a que l'immunité qui est spécifique et que ce qu'on appelle anaphylaxie (et que nous appelons paraphylaxie) est un état non spécifique produit à côté de l'immunité qui — elle — est spécifique.

3° Si la maladie du sérum et les infections étaient dues à l'anaphylatoxine (Friedberger—v. Pirquet) leur symptomatologie devrait être identique. Les manifestations cutanées par exemple dans la maladie du sérum, la rougeole, la scarlatine devraient être identiques. Or, toutes ces maladies présentent leurs caractères à part.

4° Si l'anaphylaxie et l'immunité étaient des états distincts et opposés il faudrait que les facteurs qui empêchent un état favorisent l'autre. Or, tous les facteurs qui favorisent l'immunité favorisent aussi l'anaphylaxie et le choc anaphylactique et tous les facteurs qui empêchent l'immunité empêchent aussi l'anaphylaxie et le choc anaphylactique.

5° *Le choc dit anaphylactique n'est pas un choc histaminique.* Il y a des différences capitales entre le choc paraphylactique acétylcholinique et le choc histaminique. Le premier est empêché par une injection de glucose, par le jeûne et par une première injection d'une petite dose du même antigène, le second n'est empêché ni par l'atropine, ni par le glucose ni par le jeûne. Et une petite dose d'histamine ne prémunit pas contre une dose mortelle de la même substance.

6° Pour prouver la production de l'anaphylatoxine on a fait l'expérience appelée anaphylaxie *in vitro*. On mélange *in vitro*, l'antigène, l'anticorps et l'alexine et on introduit le mélange dans la veine du cobaye neuf, qui fait un choc anaphylactique typique. On affirme alors que l'anaphylatoxine s'est produite *in vitro*. Mais personne n'a isolé cette anaphylatoxine, n'a démontré sa nature, ni son existence dans le mélange *in vitro*. Personne ne peut nier que la substance qui en dernière analyse produit le choc n'est libérée dans l'organisme même. Et tout fait supposer que c'est dans l'organisme que cette substance se produit, car, n'importe quel serait l'antigène et son anticorps spécifique employés, qu'il soit du sérum de cheval avec du sérum de lapin anti-cheval, ou des hématies de mouton avec du sérum hémolytique de lapin anti-mouton, le choc est symptomatologiquement identique. Nous verrons que cette substance n'est pas un poison nocif, l'anaphylatoxine, mais une substance physiologique qui, libérée en grande quantité, devient nocive et peut même provoquer la mort: l'*acétylcholine*.

7° L'on prétend que le phénomène anti-anaphylactique est dû à la consommation des anticorps par la première injection faite la veille avec une petite dose d'antigène. Mais nous l'obtenons encore dans les conditions suivantes. Nous injectons chez le cobaye neuf une petite quantité d'un mélange antigène-anticorps-alexine, incapable de produire un choc évident, et le lendemain une dose qui chez le témoin, produit le choc: le choc ne se produit plus.

Il ne peut pas être question dans cette expérience d'épuisement en anticorps, car les anticorps sont injectés par nous en même temps que l'antigène. Nous avons démontré que le phénomène dit anti-anaphylactique est dû à une *déchélinisation tissulaire*, c'est à dire à une consommation en cette prés substance dont provient l'acétylcholine et que nous avons appelée précholine.

8° En étudiant la symptomatologie du choc anaphylactique nous constatons que tous les phénomènes ressemblent à l'action de l'acétylcholine, qui est une substance physiologique. Pourquoi imaginer alors la production d'un poison nouveau, l'anaphylatoxine, et ne pas supposer qu'il s'agit d'une forte libération d'acétylcholine, hypothèse qui explique l'identité du choc n'importe quel serait l'antigène employé.

9° Si le choc anaphylactique est dû à la production d'une anaphylatoxine spécifique comment se fait-il que dans l'asthme anaphylactique, le syndrome est identique n'importe quel serait l'antigène qui le produit: poudre d'ipéca, émanation de cheval ou de mouton, albumines étrangères? Et comment peut-on penser à une anaphylatoxine spécifique alors que la symptomatologie de l'accès d'asthme anaphylactique est identique à celle de l'accès d'asthme produit par un réflexe, qui se conduit principalement par la voie parasympathique. Peut-on s'imaginer que l'influx parasympathique puisse libérer l'anaphylatoxine?

10° Nous injectons un mélange antigène — anticorps dans la veine de l'animal neuf. Ce mélange est immunisant et ne doit provoquer aucun phénomène pathologique chez l'animal neuf. Comment peut-on s'imaginer qu'un mélange immunisant puisse provoquer un phénomène d'hypersensibilité pour cet antigène? Dès le début on aurait dû penser que les phénomènes pathologiques qui apparaissent (le choc) ne peuvent pas être dus à une anaphylatoxine spécifique et il faut supposer qu'il y a une autre substance qui apparaît, non pas contraire à l'immunité, mais produite *à côté* du phénomène d'immunité.

11° Un antigène est capable de provoquer une maladie spécifique: la toxine tétanique produit le tétanos, la toxine diphtérique produit une maladie toxique et des lésions à part dont fait partie la paralysie du train postérieur et les lésions de la capsule surrénale, une bactérie une infection spécifique, etc. Le même antigène est capable d'immuniser et en même temps d'anaphylactiser l'organisme. L'antigène est capable par conséquent de provoquer trois états: l'immunité, l'anaphylaxie et la maladie spécifique. On a étudié séparément le mécanisme de production de chacun de ces trois états, mais il n'est nullement établi jusqu'à présent quelle est la relation entre ces états, qui sont pourtant provoqués par le même antigène et dans le même organisme. On ne peut pas se contenter de la conception soutenue actuellement qui n'explique ni la relation entre l'immunité et l'anaphylaxie, ni la relation entre l'anaphylaxie et la maladie spécifique. Von Pirquet et Friedberger ont assimilé la maladie du sérum et les infections aux phénomènes produits par le choc anaphylactique. Mais nous ne voyons pas comment une fièvre typhoïde qui dure trois semaines ou un typhus exantématique qui dure 14 jours pourrait s'expliquer par un choc

qui ne peut être que transitoire. Et d'ailleurs, nous savons que pendant la période d'état de l'infection les phénomènes anaphylactiques ne se produisent pas: il s'établit ce qu'on appelle allergie. Et d'ailleurs, comme nous l'avons dit plus haut, si ces maladies ne seraient que des phénomènes anaphylactiques, et comme le choc anaphylactique pur est toujours identique, il faudrait que la symptomatologie de la maladie du sérum ou d'un tétanos ou d'une scarlatine soit identique.

B. Conception personnelle sur le mécanisme de l'immunité, de l'anaphylaxie et des maladies spécifiques produites par les antigènes (maladie du sérum, infections, etc.).

Phylaxie, paraphylaxie (maladie non spécifique) et maladie spécifique.

Nous avons exposé pour la première fois cette hypothèse en 1931, à l'occasion de recherches que nous avons fait dans l'asthme anaphylactique et non anaphylactique. Nous avons affirmé alors que le choc anaphylactique est dû à la libération d'acétylcholine. Nous avons complété ensuite notre conception que nous exposons plus bas ¹.

L'antigène ne provoque dans l'organisme que l'immunité, ou *phylaxie*. Il n'y a pas de sensibilisation de l'organisme (anaphylaxie), il ne se produit pas d'anticorps sensibilisants. Tous les anticorps sont immunisants. L'antigène a deux actions: une *action non spécifique* qui consiste en la libération d'acétylcholine (action acétylcholinergique) et qui est commune à tous les antigènes et une *action spécifique*, différente pour chaque antigène et qui provoque la «*maladie spécifique*» (maladie du sérum si l'antigène a été du sérum étranger, tétanos si nous avons injecté de la toxine tétanique, infections s'il s'agit d'antigènes vivants). L'action non spécifique s'exerce immédiatement (c'est l'action acétylcholinergique) alors que l'action spécifique ne s'exerce qu'après une période d'incubation, pendant laquelle l'organisme tend à s'immuniser. Et l'action spécifique ne se produit que si l'immunité a été incom-

¹ D. Danielopolu — Pathogénie de l'asthme- Arch. méd. chir. appareil respiratoire. Masson 1931; Congrès de l'Asthme du Mont Dore, 1932, etc.

plète. L'immunité s'établit par la production des anticorps, qui sont tous immunisants. L'antigène libéré de l'acétylcholine et provoque la formation des anticorps, qui se forment en étroite union avec l'acétylcholine. Sans le concours de l'acétylcholine les anticorps ne peuvent pas se former. C'est ce que nous avons appelé «*anticorps choline*». Nous avons proposé ce nom pour désigner la relation étroite qui se produit dans la cellule entre l'anticorps et la précholine¹ contenue dans toutes les cellules sans pourtant soutenir que la précholine fait partie de la molécule de l'anticorps.

Nous sommes très enclins de croire que l'anticorps est le résultat d'une combinaison entre l'antigène et des produits de réaction cellulaire dont les globulines et la précholine.

L'anticorps choline possède une *fonction spécifique* qui est l'anticorps et qui est capable de fixer et rendre inoffensif l'antigène et une *fonction non spécifique* qui est la précholine. Comme leur nom l'indique la première fonction est spécifique pour chaque anticorps (qui ne peut fixer que l'antigène correspondant), alors que la seconde fonction n'est pas spécifique, étant commune à tous les anticorps choline, n'importe quel serait l'antigène qui les a provoqués. L'organisme se trouve en état d'immunité ou *phylaxie* due à la fonction anticorps et en état de ce que nous avons appelé *paraphylaxie* produite par la fonction choline. Cet état n'est qu'une hyperconcentration des tissus en précholine.² La paraphylaxie n'est que ce qu'on appelle anaphylaxie. Selon la conception classique tant la phylaxie que l'anaphylaxie sont spécifiques. Toute autre est notre conception.

L'état de phylaxie est naturellement spécifique car elle ne prémunit que contre l'antigène correspondant. Mais l'état de paraphylaxie n'est pas spécifique: il consiste toujours en une hyperconcentration en précholine des tissus, n'importe quel serait l'antigène qui ait provoqué l'état de phylaxie-paraphylaxie. Mais la paraphylaxie qui n'est pas spécifique est liée à la phylaxie qui — elle — est spécifique. Aussi les phénomènes paraphylactiques

¹ Nous avons appelé précholine la prés substance que contient tous les organes terminaux et d'où l'influx nerveux et d'autres facteurs acétylcholinergiques (qui agissent sans le concours de l'influx nerveux) libèrent l'acétylcholine active. La précholine se trouve normalement en un certain degré de concentration. A mesure que les anticorps choline augmentent la concentration en précholine s'élève aussi. En effet, les anticorps se forment avec le concours de l'acétylcholine qui est libérée en permanence par l'action acétylcholinergique de l'antigène.

(appelés jusqu'à présent anaphylactiques) tout en étant toujours identiques avec n'importe quel antigène, ne se produisent qu'à l'occasion de phénomènes phylactiques qui sont spécifiques.¹

Ainsi donc, selon notre conception ce qu'on a appelé anaphylaxie n'existe pas. Il ne s'agit pas d'un état distinct de l'immunité et opposé à elle, ni d'un état spécifique. Il s'agit d'un état non spécifique qui se produit à côté d'un état spécifique, qui est l'état de phylaxie.

C'est pour cette raison que nous avons proposé de remplacer le nom d'anaphylaxie par celui de *paraphylaxie*. L'état de paraphylaxie est un état anormal des tissus. En effet, l'hyperconcentration des tissus en précholine modifie le tonus et la réactivité des organes. Comme cette hyperconcentration en précholine des tissus est identique n'importe quel serait l'antigène en cause, nous désignons la paraphylaxie comme «*la maladie non spécifique*» provoquée par l'antigène.

Les deux schémas que nous donnons plus bas représentent, le premier la conception classique, le second notre conception personnelle.

Conception classique

Antigène	\nearrow anticorps immunisants — \nwarrow anticorps anaphylactisants	Phylaxie (spécifique)
		Anaphylaxie (spécifique)

Conception personnelle

Antigène	\rightarrow	anticorps-choline
		\downarrow \downarrow Phylaxie-paraphylaxie (spécifique) (non spécifique)

Nous examinerons ce qui se produit lorsque l'anticorps vient en contact avec l'antigène.

Selon la conception admise actuellement il se forme deux complexes: un *complexe phylactique* composé d'antigène, d'anticorps

¹ C'est ce fait qui a trompé tous les auteurs et qui les a conduits à considérer l'anaphylaxie comme spécifique. Etant toujours produite par l'acétylcholine, elle ne peut qu'être identique, n'importe quel serait l'antigène qui est en cause. Et naturellement on s'est étonné de voir un syndrome identique correspondant à un phénomène spécifique. Notre conception prouve que cette contradiction n'est qu'apparente.

immunisant et d'alexine qui défend l'organisme contre l'antigène, et un *complexe anaphylactique* composé d'antigène, d'anticorps anaphylactique et d'alexine qui libère un poison l'anaphylatoxine (qui produit le choc anaphylactique). Selon cette conception, l'organisme, en même temps qu'il est immun contre l'antigène, est hypersensible vis-à-vis du même antigène.

Selon notre conception il ne se produit qu'un seul complexe, le *complexe phylactique* ou immunisant et à côté du phénomène d'immunité, une libération d'acétylcholine qui provoque le choc dit anaphylactique, que nous appelons *choc paraphylactique acétylcholinique*. Nous connaissons deux catégories d'anticorps: anticorps qui agissent avec le concours de l'alexine (bactériolysines, cytolytines, etc) et anticorps qui agissent sans alexine (agglutinines, précipitines, antitoxines, etc). Tous les anticorps appartenant à la première ou à la seconde catégorie sont immunisants. Il n'y a pas d'anticorps anaphylactisant. Mais, le complexe phylactique formé avec les anticorps qui agissent avec le concours de l'alexine libère beaucoup d'acétylcholine à la fois (*acétylcholinogénèse explosive*) alors que le complexe phylactique formé avec les anticorps qui agissent sans alexine libère peu d'acétylcholine à la fois (*acétylcholinogénèse lente*).

La formation du complexe phylactique s'accompagne de libération d'acétylcholine. En petite quantité l'acétylcholine ne produit pas de fortes modifications dans le fonctionnement des organes, car elle s'inactive très rapidement. Mais, lorsqu'elle est libérée en forte quantité et très rapidement elle provoque un choc évident qui peut même être mortel qu'on appelle choc anaphylactique, et que nous appelons *choc paraphylactique acétylcholinique*.

Les schémas suivants représentent la conception classique et notre conception personnelle sur le mécanisme du choc dit anaphylactique.

Conception classique.

La réintroduction de l'antigène dans un organisme ayant déjà été traité par le même antigène produit deux complexes:

1° Antigène + anticorps immunisant + alexine = complexe phylactique

2° Antigène + anticorps anaphylactisant + alexine = complexe anaphylactique.

Conception personnelle.

La réintroduction de l'antigène dans un organisme ayant déjà été traité par le même antigène, produit un seul complexe: le complexe phylactique.

a) Pour les anticorps qui agissent avec le concours de l'alexine:
 Antigène + anticorps choline + alexine =
 antigène — anticorps-alexine (complexe phylactique)
 + acétylcholine active (— phénomènes paraphylactiques évidents — choc)

b) Pour les anticorps qui agissent sans alexine:
 antigène + anticorps choline = antigène — anticorps
 (complexe phylactique) + acétylcholine active
 (phénomènes paraphylactiques latents — pas de choc évident).

Ce ne sont là que de simples formules provisoires. *Mais nous pouvons affirmer avec certitude que lors de la production du complexe phylactique il se libère de l'acétylcholine.*

Nous examinerons maintenant tous les phénomènes qui peuvent se produire à la suite de l'introduction d'un antigène dans l'organisme. Nous étudierons les phénomènes dans leur ensemble essayant d'expliquer en même temps le mécanisme de l'immunité (ou phylaxie), de la paraphylaxie (ou maladie non spécifique), du choc paraphylactique acétylcholinique (accident de la maladie non spécifique) et de la maladie spécifique.

Nous étudierons les phénomènes qui se produisent lors d'une première introduction de l'antigène dans un organisme neuf et les phénomènes qui se produisent lors de la réintroduction du même antigène.

Un antigène introduit pour la première fois dans un organisme neuf peut —:

1° — ne pas s'adapter à l'organisme et ne provoquer aucune réaction

2° — provoquer un phénomène d'immunité (phylaxie) complète avec, à côté, l'état de paraphylaxie ou *maladie non spécifique*,

3° — provoquer un phénomène d'immunité incomplète avec, à côté, état de paraphylaxie (*maladie non spécifique*) et après une certaine période d'incubation la *maladie spécifique*.

Un antigène introduit dans un organisme ayant déjà reçu (un certain intervalle de temps avant) le même antigène peut:

1° — provoquer sans incubation un phénomène d'immunité complète avec choc paraphylactique acétylcholinique.

2° — provoquer sans incubation un phénomène d'immunité incomplète avec choc paraphylactique acétylcholinique suivi de maladie spécifique.

Voici, selon nous, le mécanisme de tous ces phénomènes.

Examinons d'abord toutes les éventualités qui peuvent se produire lorsqu'un antigène (sérum étranger, bactéries, etc) est introduit pour la première fois dans un organisme neuf. L'antigène est une substance étrangère qui ne peut agir qu'après adaptation à l'organisme. Un antigène peut n'avoir aucune action et l'organisme ne présenter aucune réaction. Dans ce cas, l'antigène reste un simple corps étranger, qui ne provoque aucune modification tissulaire et qui finit par s'éliminer. Il se produit ni phylaxie, ni paraphylaxie, ni maladie spécifique. C'est le cas des bactériémies passagères.

Par contre, d'autres fois, l'antigène s'adapte à l'organisme. Sa première action est l'action *acétylcholinergique*: agissant sur la cellule il libère de l'acétylcholine. Par une combinaison de l'antigène avec les produits de réaction de la cellule, donc les globulines et l'acétylcholine, naissent ce que nous avons appelé les anticorps-choline et par conséquent un état de phylaxie spécifique (due aux anticorps) et un état de paraphylaxie non spécifique (due à l'hyperconcentration des tissus en précholine). Une partie de l'antigène sert à la formation des anticorps choline, lesquels tendent à fixer l'antigène resté libre (non transformé). Grâce à leur affinité spécifique l'antigène s'unit à l'anticorps et forment ensemble le *complexe phylactique* (ou immunisant). Ce complexe est constitué de l'antigène, de l'anticorps et de l'alexine, pour les bactériolysines, les cytolytines et de l'antigène et de l'anticorps pour les antitoxines, les précipitines et les agglutinines. La formation du complexe phylactique est accompagné de libération d'acétylcholine.

Deux cas peuvent se produire. Dans un premier cas les anticorps rendent inoffensif l'antigène petit à petit, par petites fractions et l'antigène arrive à être intégralement annihilé (phénomène d'*immunité complète*). Les complexes phylactiques qui prennent ainsi naissance libèrent peu d'acétylcholine à la fois. Elle s'inactive rapidement et l'organisme n'en souffre pas d'une mani-

ère cliniquement évidente (il ne se produit pas de choc acétylcholinique évident). D'un autre côté, l'antigène étant fixé et annihilé d'une manière intégrale, il n'en reste aucune trace qui puisse exercer son action spécifique, qui puisse provoquer la maladie spécifique. L'organisme supporte par conséquent sans aucun trouble tous les phénomènes et il n'apparaît aucun phénomène clinique anormal évident.

C'est le cas d'une injection d'un sérum étranger qui ne provoque pas de maladie du sérum tardive ou d'un cobaye auquel nous avons fait une seule injection de sérum de cheval. C'est aussi le cas d'un agent infectieux qui, grâce aux phénomènes *d'immunité complète* qui se sont produits ne provoque pas d'infection.

L'organisme semble normal, mais il n'est pas normal. Il est en état de phylaxie et de paraphylaxie, la phylaxie étant provoquée par les anticorps et la paraphylaxie par la choline. L'hyperconcentration en précholine des tissus modifie le tonus des organes et augmente leur réactivité à tous les facteurs qui sont capables de libérer de l'acétylcholine (facteurs acétylcholinergiques). L'organisme est malade: c'est ce que nous appelons *«maladie non spécifique»*. Elle n'est pas spécifique, car elle est la même n'importe quel serait l'antigène.

Il est facile de le démontrer. Chez un cobaye qui a reçu une première injection de sérum de cheval, nous faisons une seconde injection dans la veine: il se produit ce qu'on appelle improprement choc anaphylactique, et que nous appelons *choc paraphylactique acétylcholinique*. Nous y reviendrons plus bas.

Nous examinerons maintenant un second cas, dans lequel l'antigène n'est pas fixé par petites fractions par l'anticorps et à un moment donné une grande quantité d'antigène est fixée à la fois, sans que l'antigène soit fixé intégralement. Il se produit alors un complexe phylactique avec forte libération d'acétylcholine, qui provoque un choc dit anaphylactique (que nous appelons *choc paraphylactique acétylcholinique*). Comme tout l'antigène n'a pas été fixé et il reste une partie libre, cette partie *sous l'influence du choc paraphylactique acétylcholinique ainsi produit* exerce son action spécifique et provoque la maladie spécifique¹. Dans ce cas par

¹ Nous ne savons pas de quelle manière le choc paraphylactique acétylcholinique favorise l'action spécifique de l'antigène resté libre et déclenche la maladie spécifique. Mais nos observations ne laissent plus aucun doute qu'il existe un rapport de filiation entre le choc et la maladie spécifique.

conséquent, une première fraction de l'antigène (A_1) sert à la formation de l'anticorps choline, une seconde fraction (A_2) entre dans la formation du complexe phylactique et une troisième fraction (A_3 qui reste libre) provoque la maladie spécifique. Il s'agit dans ce cas d'un phénomène d'immunité incomplète, car tout l'antigène n'a pas été fixé dans le complexe phylactique (fig. 1).

C'est le cas d'une seule injection de sérum qui a provoqué, après 8 à 12 jours la maladie du sérum. Le début de la maladie du sérum est caractérisé par des phénomènes acétylcholiniques (bradycardie) hypotension, leucopénie avec éosinophilie et mononucléose) suivis de phénomènes dus à l'action spécifique de l'antigène resté libre (phénomènes cutanés et articulaires, hyperthermie, névrites, etc). C'est aussi le cas d'un agent infectieux qui, après

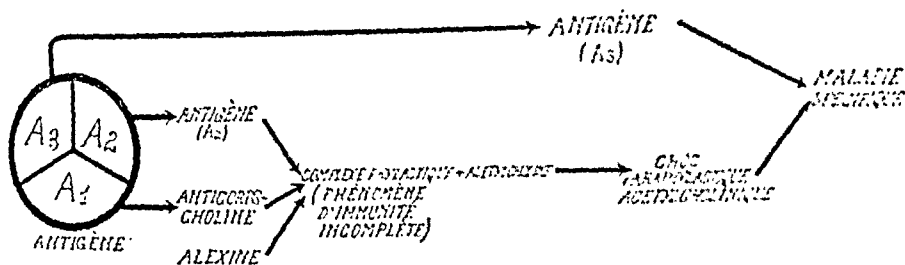


Fig. 1.

une certaine période d'incubation, a provoqué une infection, caractérisée aussi au début par des phénomènes acétylcholiniques suivis de phénomènes spécifiques.

Dans ce deuxième cas aussi nous trouvons pendant l'incubation un état de phylaxie et de paraphylaxie, mais l'état de phylaxie n'étant pas suffisant, pour fixer tout l'antigène, une partie de l'antigène reste libre et provoque la maladie spécifique.

Entre le premier et le second cas nous trouvons deux grandes différences. Première différence: dans le premier cas l'antigène est fixé par petites portions et ne donne pas de choc, dans le second la fixation se fait d'un coup. Seconde différence: dans le premier cas l'antigène est fixé intégralement et dans le second la fixation n'est pas intégrale. Il semble que pour que le phénomène d'immunité soit complet et fixe l'antigène, il est nécessaire que la fixation se fasse par petites portions et que lorsque la formation du complexe phylactique libère beaucoup d'acétylcholine et provo-

que un choc, l'antigène resté libre est capable d'exercer son action spécifique et de provoquer la maladie spécifique.

La différence signalée plus haut peut être en relation avec la proportion réciproque des anticorps qui agissent sans alexine et de ceux qui agissent avec le concours de l'alexine. Mais ce n'est là qu'une simple supposition.

Examinons maintenant l'évolution de la maladie spécifique. Une fois la maladie spécifique commencée, la lutte pour la défense contre l'antigène qui l'a provoquée recommence. L'antigène continue son action acétylcholinergique et donne naissance à de nouveaux anticorps-choline. Mais les conditions sont plus défavorables car la formation des anticorps nécessite de l'acétylcholine, et cette dernière est en partie consommée par les phénomènes spécifiques eux-mêmes. Nous considérons, en effet, l'acétylcholine comme le facteur vital de première importance dans l'organisme qui préside au métabolisme cellulaire et à toutes les fonctions vitales de la cellule. Pendant la maladie spécifique le métabolisme cellulaire s'accroît et il se produit une grande consommation d'acétylcholine.

Deux phénomènes évoluent en même temps: d'un côté l'action spécifique qui se produit avec consommation d'acétylcholine et qui tend à abaisser la concentration en précholine des tissus, de l'autre l'action acétylcholinergique de l'antigène qui tend à l'augmenter. Pendant la période d'état de la maladie c'est le premier phénomène qui prédomine et la courbe acétylcholinique reste basse. C'est ce qui explique dans une infection aiguë la tachycardie, qui, d'après nos recherches faites avec l'éprouve de l'atropine et de l'orthostatisme est due à une diminution en concentration de l'acétylcholine. Mais, si la maladie guérit, le deuxième phénomène s'intensifie et arrive à prédominer sur le premier. Pendant la période d'état il se produit continuellement des anticorps. Leur formation, difficile au commencement par manque d'acétylcholine, se fait rapidement et en grande quantité vers sa fin. Pendant la période d'état les anticorps tendent à fixer petit à petit l'antigène. Vers la fin la concentration en anticorps arrivant à un haut degré, ils fixent tout l'antigène resté libre et les phénomènes spécifiques disparaissent (par manque d'antigène libre). La terminaison de la maladie spécifique peut se faire d'une manière brusque, comme cela se passe dans une pneumonie où la défervescence se

produit en quelques heures. La déservescence brusque d'une pneumonie n'est qu'un choc paraphylactique acétylcholinique dû à la libération d'acétylcholine faite à l'occasion de la formation d'un complexe phylactique dans lequel tout l'antigène est fixé. Alors que le début d'une pneumonie est marqué par un phénomène d'immunité incomplète qui donne un choc paraphylactique acétylcholinique suivi de phénomènes spécifiques, la déservescence est due à la formation d'un phénomène d'immunité complet, dans lequel tout l'antigène est fixé, caractérisé par des phénomènes acétylcholiniques purs. Ils ne sont pas suivis de phénomènes spécifiques car il ne reste aucune trace d'antigène libre qui puisse les produire.

C'est par une augmentation de la courbe acétylcholinique vers la fin de l'infection que nous avons expliqué la bradycardie, la

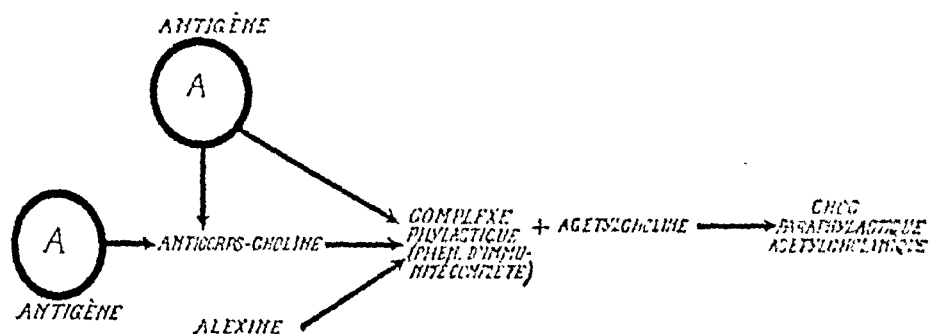


Fig. 2.

leucopénie et l'éosinophilie de la convalescence d'une maladie infectieuse.

Dans le cas des antigènes inertes ce n'est que l'action acétylcholinergique de l'antigène qui intervient dans la production de l'acétylcholine. Dans le cas des antigènes vivants nous attribuons un rôle important aussi à l'acide p-amino-benzoïque qui dans nos recherches s'est montré aussi acétylcholinergique. Nous savons que ce produit joue un rôle important dans le métabolisme et la multiplication des microbes. Nous lui attribuons aussi un rôle dans l'acétylcholinogénèse nécessaire à la formation des anticorps.

Nous avons examiné jusqu'ici ce que peut produire un antigène introduit dans l'organisme une première et seule fois. Nous examinerons maintenant ce qui se passe lorsque dans un organisme en état de phylaxie et de paraphylaxie nous introduisons une nouvelle dose du même antigène.

Nous avons dit que la première introduction d'antigène provoque un état de phylaxie-paraphylaxie dû à la présence d'anticorps-choline. Ces derniers sont toujours en excès.

Trois cas peuvent se présenter. Dans un premier cas l'organisme est bien immunisé et l'antigène réintroduit dans l'organisme est fixé intégralement et rendu inoffensif. Il se produit un phénomène phylactique complet avec libération d'acétylcholine qui provoque un choc paraphylactique acétylcholinique évident. (fig. 2) C'est ce qu'on appelle choc anaphylactique. C'est le cas du cobaye en état de paraphylaxie qui fait un choc paraphylactique acétylcholinique mortel lors de la seconde injection du même antigène. Mais si la libération d'acétylcholine ne se fait pas à cette occasion d'une manière assez rapide il n'apparaît pas de choc évident.

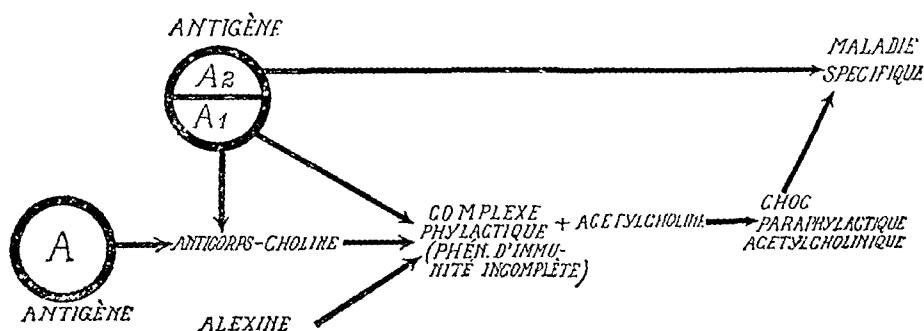


Fig. 3.

C'est le cas d'un organisme parfaitement immunisé contre une infection et chez lequel est introduit le même microbe.

Dans tous ces cas il n'apparaît pas de maladie spécifique, car tout l'antigène réintroduit a été capturé par les anticorps. Mais il se peut que le phénomène d'immunité soit incomplet et qu'une partie de l'antigène réintroduit reste libre. Dans ce cas une première fraction de l'antigène (A_1) sert à la formation du complexe phylactique, qui libère de l'acétylcholine et provoque le choc paraphylactique qui à son tour favorise l'action spécifique de la seconde fraction d'antigène (A_2) qui reste encore libre et déclenche la maladie spécifique (fig. 3). Tandis que dans le premier cas, dans lequel tout l'antigène nouvellement introduit a été fixé, il ne se produit que des phénomènes acétylcholiniques, dans ce dernier cas les phénomènes acétylcholiniques sont suivis de phénomènes spécifiques.

Dans tous ces cas le phénomène se produit immédiatement, sans incubation. En effet, l'incubation n'est nécessaire que pour la formation des anticorps, qui dans ces cas se trouvent déjà en excès dans l'organisme.

Dans notre manière de voir nous n'admettons par conséquent pas l'existence de l'anaphylaxie. Il n'y a pas de sensibilisation de l'organisme, et l'antigène ne produit que le phénomène d'immunité. Mais ce phénomène étant provoqué par les anticorps qui ne peuvent se produire qu'avec le concours de la précholine tissulaire, cette dernière augmente en concentration dans les tissus. Cet état n'est nullement un état contraire à l'immunité, mais un état produit à côté de l'immunité et dépendant d'elle. Il n'y a pas par conséquent d'anaphylaxie, mais ce que nous avons appelé paraphylaxie. Sur un organisme en état de phylaxie et d'hyperconcentration tissulaire (paraphylaxie) toute trace d'antigène qui est resté libre dans l'organisme ou que nous avons réintroduit provoque la formation d'un complexe phylactique avec libération d'acétylcholine qui provoque le choc qu'on a appelé anaphylactique. Mais ce choc n'a rien à faire avec une sensibilisation quelconque de l'organisme. Il s'agit d'un phénomène produit à côté du complexe phylactique, un déchet de ce complexe. C'est pour cette raison que nous avons remplacé le terme de choc anaphylactique avec celui de *choc paraphylactique acétylcholinique*.

Le choc n'est pas spécifique, car il est identique n'importe quel aurait été l'antigène qui a provoqué l'immunité à côté de laquelle l'état de paraphylaxie s'est formée, mais il se produit à côté du complexe phylactique qui — celui là — est spécifique car il ne peut prendre naissance qu'entre l'antigène et son anticorps spécifique correspondant. Ce qu'on a appelé *anaphylaxie passive*, n'est qu'un choc paraphylactique acétylcholinique produit à côté d'un phénomène d'immunité passive et ce qu'on a appelé *anaphylaxie in vitro* n'est qu'un choc paraphylactique acétylcholinique provoqué in vivo avec un mélange immunisant fait in vitro.

Quant au phénomène appelé anti-anaphylaxie, il est dû — en grande partie — à ce que nous avons appelé *décholinisation tissulaire*. Lorsque chez un animal traité par un antigène nous introduisons une petite dose du même antigène, il se produit un complexe phylactique avec libération d'acétylcholine. Il y a en même temps consommation d'anticorps et consommation de précholine (décho-

linisation tissulaire). Si nous injectons le lendemain une dose du même antigène qui chez le témoin est mortelle, elle ne produit plus la mort, car le choc paraphylactique est provoqué par la libération d'acétylcholine et la source d'où est tirée cette substance est appauvrie par la formation du complexe phylactique antérieur.

Nous exposerons dans les mémoires suivants les preuves et les arguments qui nous ont conduits à cette conception.

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